Abstract:
The present invention is related to a YB-1 dependent virus for use in a method for killing tumor stem cells of a tumor.
Method for killing tumor stem cells

The present invention is related to a virus for use in a method for killing tumor stem cells of a tumor, a virus for use in the treatment of a tumor, a virus for use in a method for intratumorally administering a virus, a nucleic acid coding for the same, a pharmaceutical composition comprising such virus, a pharmaceutical composition comprising such nucleic acid and methods for the killing of tumor stem cells and a method for intratumorally administering a virus.

Every year about 350,000 people develop a malignant tumour in the Federal Republic of Germany. Less than 50% of these patients can expect a definitive cure. Apart from surgical removal and radiation, chemotherapy using cytostatics is the most common form of treatment of cancer for the time being. The antineoplastically active substances used in connection therewith are, in principle, effective against all cells of the organism, however, tumour cells are more prone to chemosensitivity due to their increased proliferation rate. Good therapeutic results can be obtained for various tumour entities such as juvenile lymphatic leukaemia, some lymphoma and testicular carcinoma. However, these tumours represent only 10% of all malignant diseases. Most of the solid tumours do not respond or respond only weakly to a treatment using various cytostatics. This is particularly true for carcinoma derived from kidney, colon, pancreas and liver as well as melanoma and brain tumours. Additionally, e.g., mammary carcinoma, ovarian carcinoma and prostate carcinoma initially respond well to cytostatic treatment, however, get insensitive to the cytostatics used in the course of the therapeutical cycles. In accordance therewith, studies show that there is a high correlation between P-glycoprotein expression and occurrence of metastases. Although significant progress has been made in the previous years in the development of established therapeutic concepts such as chemotherapy and radiation therapy, all in all the treatment of solid tumours in particular is still not satisfactory.

Apart from the classical therapeutic concept for the treatment of tumors such as surgery, chemotherapy and radiotherapy the use of replication selective oncolytic viruses provides for a new platform for the treatment of tumors. In connection therewith a selective intratumor
replication of a viral agent is initiated which results in virus replication, lysis of the infected tumor cell and spreading of the virus to adjacent tumor cells. As the replication capabilities of the virus is limited to tumor cells, normal tissue is spared from replication and thus from lysis by the virus. Various forms of oncolytic viruses have been described so far.

One example for such an adenovirus is dll520 (Onyx-015) which has been successfully used in clinical phases I and II (Khuri, F. et al. Nature Medicine 6, 879-885, 2000). Onyx-015 is an adenovirus having a completely deleted ElB-55kDa gene. The complete deletion of the ElB55kDa protein of the adenovirus is based on the discovery that replication and thus lysis of cells is possible with an adenoviral vector having a p53 deficiency (Kirn, D. et al., Proc. Am. Soc. Clin. Oncol. 17, 391a, 1998), whereby normal cells are not harmed. More particularly, the ElB-55kDa gene product is involved in the inhibition of p53, the transport of viral mRNA and the switching off the protein synthesis of the host cell. The inhibition of p53 occurs via formation of a complex consisting of p53 and the adenoviral coded ElB-55kDa protein and/or a complex consisting of ElB-55kDa and E4orf6. p53, coded by TP53, is the starting point for a complex regulatory mechanism (Zambetti, G.P. et al., FASEB J. 7, 855-865, 1993), which results, among others, in an efficient inhibition of the replication in the cell of viruses like adenovirus. The gene TP 53 is deleted or mutated in about 50% of all human tumors which results in the absence of - desired - apoptosis due to chemotherapy or radiation therapy resulting in an usually unsuccessful tumor treatment.

A further concept of tumorlytic adenoviruses is based on the discovery that if the E1A protein is present in a specific deleted form or comprises one or several mutations, which do not affect the binding of Rb/E2F and/or pl07/E2F and/or pl30/E2F, such adenovirus will not induce the entry of the infected cells into the S phase and will be capable of replicating in tumor cells which do not have a functional Rb protein. Additionally, the E1A protein can be deleted at the N-terminus and comprise one or several mutations in the region of amino acid positions 1 to 76 of the E1A proteins, respectively, in order to inhibit the binding of E1A to p300 and thus to provide for a selective replication in tumor cells. These approaches are described in an exemplary manner in European patent EP 0 931 830. Examples for such viruses are AdA24, dll922 - 947, E1Ad/01/07 and CB016 (Howe, J. A. et al., Molecular Therapy 2, 485-495, 2000; Fueyo, J. et al., Oncogene 19, 2-12, 2000; Heise, C. et al., Nature
Medicine 6, 11341-139, 2001; Balague, C. et al., J. Virol. 75, 7602-7611, 2001). These adenoviral systems for oncolysis known in the prior art thus comprise distinct deletions in the E1A protein, whereby such deletions had been made under the assumption that a functional Rb protein and complexes consisting of inactive Rb protein and E2F, respectively, would block an efficient in vivo replication and in order to provide an adenoviral replication in vivo in Rb-negative/mutated cells only. These adenoviral systems according to the prior art are based on E1A in order to control in vivo replication using the early E2 promoter (engl. E2 early promoter) and free E2F (Dyson, N. Genes & Development, 12, 2245-2262, 1998).

A further group of oncolytic adenoviruses which are described in the art are those which replicate in a YB-1 dependent manner. Such oncolytic adenoviruses are those described in international patent applications WO 03/099859 and WO 2004/035616.

The oncolytic adenoviruses as described in WO 03/099859 are replication deficient in cells which do not have YB-1 in the nucleus, and code for an oncogene or oncogene protein which transactivates at least one adenoviral gene in YB-1 nucleus positive cells such as ElB55kDa, E4orf6, E4orf3 and E3ADP.

The oncolytic adenoviruses as described in WO 2004/035616 are expressing a first protein which is selected from the group comprising an E1B protein and an E4 protein, prior to a second protein which is selected from the group comprising an E1A-protein.

A still further form of tumorlytic adenoviral systems is based on the use of selective promoters for specifically expressing the viral oncogene E1A which provides for a selective replication in tumor cells (Rodriguez, R. et al., Cancer Res. 57, 2559-2563, 1997).

A particular problem in the treatment of tumors and thus cancer is the existence of tumor stem cells. Such tumor stem cells are present in factually each and any tumor and solid tumor in particular and give rise to a relapse after the killing or removal of tumor cells different from tumor stem cells (non-tumor stem cells).
The problem underlying the present invention was to provide a means for the killing of tumor stem cells, and in particular the *in vivo* and/or *in situ* killing of such tumor stem cells. The problem underlying the present invention was also to provide means for treating a tumor and thus cancer whereby the tumor contains or arises from tumor stem cells. Another problem underlying the present invention was to provide means which allow the use of viruses and more specifically of adenoviruses in the treatment of a tumor and thus cancer by means of intratumoral administration of such virus.

These and other problems are solved by the subject matter of the attached independent claims. Preferred embodiments may be taken from the attached independent claims.

More specifically, the problems underlying the present invention is solved in a first aspect by a YB-1 dependent virus for use in a method for killing tumor stem cells of a tumor.

In an embodiment of the first aspect the tumor stem cells are part of a tumor.

In an embodiment of the first aspect the tumor stem cells are part of a hypoxic area of the tumor or are contained in a hypoxic area of the tumor.

In an embodiment of the first aspect the virus is administered to the tumor, preferably into a hypoxic area, more preferably into the hypoxic area of which the tumor stem cells are part of or into the hypoxic area in which the tumor stem cells are contained.

More specifically, the problems underlying the present invention is solved in a second aspect by a YB-1 dependent virus for use in the treatment of a tumor, wherein the treatment comprises the killing of tumor stem cells.

In an embodiment of the second aspect the tumor stem cells are related to the tumor to be treated. As preferably used herein, a tumor stem cell is associated with the tumor if the tumor stem cells form part of the tumor or the tumor is derived from the tumor stem cell.

In an embodiment of the second aspect the tumor stem cells are part of a tumor.
In an embodiment of the second aspect the tumor stem cells are part of a hypoxic area of the tumor or are contained in a hypoxic area of the tumor.

In an embodiment of the second aspect the virus is administered to the tumor, preferably into the hypoxic area.

More specifically, the problems underlying the present invention is solved in a third aspect by a YB-1 dependent virus for use in a method for intratumorally administering a virus.

In an embodiment of the third aspect the virus to be administered is the YB-1 dependent virus.

In an embodiment of the third aspect the virus is administered to a hypoxic area of the tumor.

In an embodiment of the first, second and third aspect the virus is an adenovirus.

In an embodiment of the first, second and third aspect the virus is an adenovirus different from wild type adenovirus.

In an embodiment of the first, second and third aspect the virus requires YB-1 for replication.

In an embodiment of the first, second and third aspect the virus replicates in cells containing YB-1.

In an embodiment of the first, second and third aspect the YB-1 is contained in the nucleus of the cells, preferably independent of the cell cycle.

In an embodiment of the first, second and third aspect YB-1 is contained in the cytoplasm of the cells.

In an embodiment of the first, second and third aspect the YB-1 is deregulated YB-1.
In an embodiment of the first, second and third aspect the deregulated YB-1 is acetylated or phosphorylated YB-1.

In an embodiment of the first, second and third aspect the deregulated YB-1 is YB-1 which is over-expressed in the cell, preferably a tumor cell and more preferably a tumor stem cell.

In an embodiment of the first, second and third aspect the YB-1 is over-expressed in the cell compared to a cell of the same type but not being a tumor cell.

In an embodiment of the first, second and third aspect the tumor stem cells contain YB-1.

In an embodiment of the first, second and third aspect the YB-1 is contained in the nucleus of the tumor stem cells, preferably independent of the cell cycle.

In an embodiment of the first, second and third aspect the YB-1 is contained in the cytoplasm of the tumor stem cells.

In an embodiment of the first, second and third aspect the YB-1 is deregulated YB-1.

In an embodiment of the first, second and third aspect the deregulated YB-1 is acetylated or phosphorylated YB-1.

In an embodiment of the first, second and third aspect the deregulated YB-1 is YB-1 which is over-expressed in the cell, preferably a tumor cell and more preferably a tumor stem cell.

In an embodiment of the first, second and third aspect the YB-1 is over-expressed in the tumor stem cell compared to a cell of the same type but not being a tumor cell, preferably not being a tumor stem cell.
In an embodiment of the first, second and third aspect the virus does not replicate in cells not containing YB-1, preferably YB-1 as defined in any of the preceding claims, but replicates in cells containing YB-1, preferably YB-1 as defined in any of the preceding claims.

In an embodiment of the first, second and third aspect the hypoxic region is a region where the oxygen partial pressure is equal to or less than 4% $pO_2$, preferably equal to or less than 2% $pO_2$ and more preferably equal to or less than 1% $pO_2$.

In an embodiment of the first, second and third aspect the virus is an oncolytic adenovirus.

In an embodiment of the first, second and third aspect the virus, preferably an adenovirus, is replication deficient in cells which lack YB-1 in the nucleus, and whereby the virus encodes an oncogene or oncogene product, in particular an oncogene protein, which transactivates at least one viral gene, preferably an adenoviral gene, whereby the gene is selected from the group comprising ElB55kDa, E4orf6, E4orD and E3ADP.

In an embodiment of the first, second and third aspect the adenovirus replicates in cells which have YB-1 in the nucleus.

In an embodiment of the first, second and third aspect the viral oncogene protein is E1A and/or the oncogene is the gene coding for E1A and/or the oncogene protein E1A.

In an embodiment of the first, second and third aspect the viral oncogene protein E1A is capable of binding a functional Rb tumor suppressor gene product.

In an embodiment of the first, second and third aspect the viral oncogene protein E1A is incapable of binding a functional Rb tumor suppressor gene product.

In an embodiment of the first, second and third aspect the viral oncoprotein E1A does not induce the localisation of YB-1 into the nucleus.
In an embodiment of the first, second and third aspect the medicament is for patients whose cells are Rb positive or Rb negative.

In an embodiment of the first, second and third aspect the cells are Rb negative and the cell nucleus is YB-1 positive, preferably YB-1 positive in the nucleus independent from the cell cycle.

In an embodiment of the first, second and third aspect the cells are p53 positive or p53 negative.

In an embodiment of the first, second and third aspect the oncogene protein exhibits one or several mutations or deletions compared to the wildtype oncogene protein E1A, whereby the deletion is preferably one selected from the group comprising deletions of the CR3 stretches and deletions of the N-terminus and deletions of the C-terminus.

In an embodiment of the first, second and third aspect the E1A oncogene protein is capable of binding to Rb.

In an embodiment of the first, second and third aspect the oncogene protein comprises one or several mutations or deletions compared to the wildtype oncogene protein, whereby the deletion is preferably a deletion in the CR1 region and/or CR2 region.

In an embodiment of the first, second and third aspect the oncogene protein E1A is incapable of binding to Rb.

In an embodiment of the first, second and third aspect the viral oncogene protein, preferably E1A, is under the control of a tissue- and/or tumor-specific promoter.

In an embodiment of the first, second and third aspect the virus, particularly the adenovirus, codes for YB-1.
In an embodiment of the first, second and third aspect YB-1 is under the control of a tissue-specific and/or tumor-specific promoter.

In an embodiment of the first, second and third aspect the virus, preferably the adenovirus, codes for at least one protein, whereby the protein is selected from the group comprising E4orf6, E4orf3, ElB55k and adenoviral E3ADP protein.

In an embodiment of the first, second and third aspect the cells comprise YB-1 in the nucleus, preferably that the cells forming the tumor or part thereof have YB-1 in the nucleus.

In an embodiment of the first, second and third aspect the tumor comprises YB-1 in the nucleus after induction of the transport of YB-1 into the nucleus.

In an embodiment of the first, second and third aspect the transport of YB-1 into the nucleus is triggered by at least one measure selected from the group comprising irradiation, administration of cytostatics and hyperthermia.

In an embodiment of the first, second and third aspect the measure is applied to a cell, an organ or an organism, preferably an organism in need thereof, more preferably an organism suffering from said disease.

In an embodiment of the first, second and third aspect the adenovirus is selected from the group comprising AdA24, dl922-947, E1Ad/01/07, dil 19/1 131, CB 016, dl520 and viruses lacking an expressed viral oncogene which is capable of binding a functional Rb tumor suppressor gene product.

In an embodiment of the first, second and third aspect the adenovirus is designed such that the replication is controlled by YB-1 through the activation of the E2-late promoter, preferably the activation is predominantly controlled through the activation of the E2-late promoter.

In an embodiment of the first, second and third aspect the virus comprises a nucleic acid coding for a transgene.
In an embodiment of the first, second and third aspect the virus comprises the translation and/or transcription product of a transgene.

In an embodiment of the first, second and third aspect the nucleic acid comprises a transgene or a nucleic acid coding for a transgene.

In an embodiment of the first, second and third aspect the transgene is selected from the group comprising prodrug genes, cytokines and genes for cytokines, apoptosis-inducing genes, tumor suppressor genes, genes for metalloproteinase inhibitors and genes for angiogenesis inhibitors.

In an embodiment of the first, second and third aspect the transgene is selected from the group comprising nucleic acids for siRNA, for aptamers, for antisense molecules and for ribozymes, whereby the siRNA, the aptamer, the antisense molecule and/or the ribozyme are targeting a target molecule.

In an embodiment of the first, second and third aspect the target molecule is selected from the group comprising resistance relevant factors, anti-apoptosis factors, oncogenes, angiogenesis factors, DNA synthesis enzymes, DNA repair enzymes, growth factors, receptors for growth factors, transcription factors, metalloproteinases, preferably matrix metalloproteinase kinases, and plasminogen activator of the urokinase type.

In an embodiment of the first, second and third aspect the virus is E1A12S positive but lacking a functionally active E1A13S.

In an embodiment of the first, second and third aspect the virus, preferably the adenovirus, expresses a first protein which is selected from the group comprising an E1B protein and an E4 protein, prior to a second protein which is selected from the group comprising an E1A-protein.
In an embodiment of the first, second and third aspect the first protein is an E1B protein, preferably an ElB55kd protein.

In an embodiment of the first, second and third aspect the first protein is an E4 protein, preferably an E4orf6 protein.

In an embodiment of the first, second and third aspect the first protein is a combination of E1B protein and E4 protein, preferably a combination of ElB55kD protein and E4orf6 protein.

In an embodiment of the first, second and third aspect the E1A protein is an E1A12S protein.

In an embodiment of the first, second and third aspect the virus comprises at least one nucleic acid coding for a protein which is selected from the group comprising E1B proteins, E4 proteins and E1A proteins, whereby the at least one protein is under the control of a promoter which is different from the promoter controlling the expression of the protein in a wildtype adenovirus.

In an embodiment of the first, second and third aspect the at least one protein is an E1B protein, preferably an ElB55kD protein.

In an embodiment of the first, second and third aspect the at least one protein is an E4 protein, preferably an E4orf6 protein.

In an embodiment of the first, second and third aspect the at least one protein is an E1A protein, preferably an E1A12S protein.

In an embodiment of the first, second and third aspect the at least one protein is a combination of E1B protein and E4 protein, preferably a combination of ElB55kD protein and E4orf6 protein.
In an embodiment of the first, second and third aspect the at least one protein is a combination of E1B protein and E1A protein, preferably a combination of E1B55kD protein and E1A12S protein.

In an embodiment of the first, second and third aspect the at least one protein is a combination of E4 protein and E1A protein, preferably a combination of E4orf6 protein and E1A12S protein.

In an embodiment of the first, second and third aspect the at least one protein is a combination of E1B protein, E4 protein and E1A protein, preferably a combination of E1B55kD protein, E4orf6 protein and E1A12S protein.

In an embodiment of the first, second and third aspect the expression of the E1B protein is controlled by a promoter, whereby the promoter is selected from the group comprising tumor-specific promoters, organ-specific promoters, tissue-specific promoters, heterologous promoters and adenoviral promoters, whereby the adenoviral promoter is different from the E1B promoter.

In an embodiment of the first, second and third aspect the expression of the E4 protein is controlled by a promoter, whereby the promoter is selected from the group comprising tumor-specific promoters, organ-specific promoters, tissue-specific promoters, heterologous promoters and adenoviral promoters, whereby the adenoviral promoter is different from the E4 promoter.

In an embodiment of the first, second and third aspect the adenoviral promoter is the E1A promoter.

In an embodiment of the first, second and third aspect the expression of the E1A protein is controlled by a promoter, whereby the promoter is selected from the group comprising tumor-specific promoters, organ-specific promoters, tissue-specific promoters, heterologous promoters and adenoviral promoters, whereby the adenoviral promoter is different from the E1A promoter.
In an embodiment of the first, second and third aspect the expression of the E1A protein is YB-1 controlled or can be regulated by YB-1.

In an embodiment of the first, second and third aspect the promoter controlling the expression of the E1A protein is the adenoviral E2 late promoter.

In an embodiment of the first, second and third aspect the E4 protein, preferably the E4orf6 protein, and the E1B protein, preferably the E1B55kd protein, are under the control of the same or a common promoter.

In an embodiment of the first, second and third aspect the virus provides YB-1 in the nucleus through at least one adenoviral protein or mediates the provision of YB-1 in the nucleus through at least one adenoviral protein, whereby preferably the adenoviral protein is different from E1A.

In an embodiment of the first, second and third aspect the virus provides YB-1 for adenoviral replication through at least one adenoviral protein or mediates the provision of YB-1 for adenoviral replication through at least one adenoviral protein, whereby preferably the adenoviral protein is different from E1A.

In an embodiment of the first, second and third aspect the adenoviral protein is a complex of E4orf6 and E1B55kd.

In an embodiment of the first, second and third aspect the nucleic acid of the adenovirus comprises at least one functionally inactive adenoviral region, whereby the region is selected from the group comprising the E1 region, the E3 region, the E4 region and combinations thereof.

In an embodiment of the first, second and third aspect the region is the E1 region.

In an embodiment of the first, second and third aspect the region is the E3 region.
In an embodiment of the first, second and third aspect the region is the E4 region.

In an embodiment of the first, second and third aspect the region comprises the E1 region, the E3 region and the E4 region.

In an embodiment of the first, second and third aspect the virus comprises at least one expression cassette, whereby the expression cassette comprises at least one promoter and a nucleic acid coding for an adenoviral protein, whereby the adenoviral protein is an E1B protein, preferably an E1B55kD protein.

In an embodiment of the first, second and third aspect the promoter is different from the E1B promoter.

In an embodiment of the first, second and third aspect the promoter is selected from the group comprising tumor-specific promoters, organ-specific promoters, tissue-specific promoters, heterologous promoters and adenoviral promoters, whereby the promoter is different from the E1B promoter.

In an embodiment of the first, second and third aspect the virus comprises at least one expression cassette, whereby the expression cassette comprises at least one promoter and a nucleic acid coding for an adenoviral protein, whereby the adenoviral protein is an E4 protein, preferably an E4orf6 protein.

In an embodiment of the first, second and third aspect the promoter is different from the E4 promoter.

In an embodiment of the first, second and third aspect the promoter is selected from the group comprising tumor-specific promoters, organ-specific promoters, tissue-specific promoters, heterologous promoters and adenoviral promoters, whereby the adenoviral promoter is different from the E4 promoter.
In an embodiment of the first, second and third aspect the promoter is the E1A promoter.

In an embodiment of the first, second and third aspect the virus comprises at least one expression cassette, whereby the expression cassette comprises at least one promoter and a nucleic acid coding for an adenoviral protein, whereby the adenoviral protein is an E1A protein, preferably an E1A12S protein.

In an embodiment of the first, second and third aspect the promoter is different from the E1A promoter.

In an embodiment of the first, second and third aspect the promoter is selected from the group comprising tumor-specific promoters, organ-specific promoters, tissue-specific promoters, heterologous promoters and adenoviral promoters.

In an embodiment of the first, second and third aspect the adenovirus comprises a nucleic acid, whereby the nucleic acid codes for YB-1.

In an embodiment of the first, second and third aspect the nucleic acid coding for YB-1 is under the control of a promoter, whereby the promoter is preferably the E2 late promoter.

In an embodiment of the first, second and third aspect the nucleic acid coding for YB-1 is under the control of a promoter, whereby the promoter is YB-1 dependent and YB-1 controlled, respectively.

In an embodiment of the first, second and third aspect the nucleic acid coding for YB-1 is part of the expression cassette comprising a nucleic acid coding for an E1A protein, preferably a nucleic acid coding for an E1A12S protein.

In an embodiment of the first, second and third aspect the nucleic acid coding for the E1A protein is separated from the nucleic acid coding for YB-1 through an IRES sequence.
In an embodiment of the first, second and third aspect the nucleic acid coding for the E4 protein, preferably the E4orf6 protein, and the nucleic acid coding for the E1B protein, preferably the E1B55kD protein, are contained in an expression cassette, whereby preferably the two coding sequences are separated through an IRES sequence.

In an embodiment of the first, second and third aspect the promoter of the expression cassette is selected from the group comprising tumor-specific promoters, organ-specific promoters, tissue-specific promoters, heterologous promoters and adenoviral promoters, whereby the adenoviral promoter is different from the E4 promoter and different from the E1B promoter, preferably different from the wildtype E4 promoter and different from the wildtype E1B promoter.

In an embodiment of the first, second and third aspect the virus comprises an expression cassette comprising a promoter and a nucleic acid sequence, whereby the nucleic acid sequence is selected from the group comprising aptamers, ribozymes, aptazymes, antisense molecules and siRNA.

In an embodiment of the first, second and third aspect the virus comprises an expression cassette comprising a promoter and a nucleic acid sequence, whereby the nucleic acid sequence is a coding nucleic acid, whereby the nucleic acid codes for a molecule which is selected from the group comprising peptides, polypeptides, proteins, anticalines, antibodies and antibody fragments.

In an embodiment of the first, second and third aspect the virus comprises an expression cassette, whereby the expression cassette comprises a promoter and a nucleic acid sequence, whereby the nucleic acid sequence is selected from the group comprising apoptosis inducing genes, prodrug genes, protease inhibitors, tumor suppressor genes, cytokines and angiogenesis inhibitors.

In an embodiment of the first, second and third aspect the virus is a recombinant adenovirus.

In an embodiment of the first, second and third aspect that the virus is an adenovirus mutant.
In an embodiment of the first, second and third aspect the virus is replication deficient.

In an embodiment of the first, second and third aspect the virus is capable of replicating in cells comprising deregulated YB-1 or having YB-1 in the nucleus.

In an embodiment of the first, second and third aspect the cells contain YB-1 in the nucleus independent of the cell cycle.

In an embodiment of the first, second and third aspect the tumor is a solid tumor.

More specifically, the problems underlying the present invention is solved in a fourth aspect by a nucleic acid coding for a virus as defined in any of the first, second and third aspect and any embodiment thereof, for use in any method as defined in connection with any one of the first, second or third aspect and any embodiment thereof.

More specifically, the problems underlying the present invention is solved in a fifth aspect by a vector comprising the nucleic acid according to the fourth aspect.

More specifically, the problems underlying the present invention is solved in a sixth aspect by a cell comprising a nucleic acid according to the fourth aspect or a vector according to fifth aspect.

In an embodiment of the sixth aspect the cell is different from a human or a human embryonic stem cell.

More specifically, the problems underlying the present invention is solved in a seventh aspect by a pharmaceutical composition comprising a virus according to any one of the first, second or third aspect or any embodiment thereof.

More specifically, the problems underlying the present invention is solved in an eighth aspect by a pharmaceutical composition comprising a nucleic acid according to the fourth aspect.
More specifically, the problems underlying the present invention is solved in a ninth aspect by a method for the killing of tumor stem cells comprising the administration of a virus according to any of the first, second or third aspect or any embodiment thereof to a tumor containing tumor stem cells or suspected of containing tumor stem cells.

More specifically, the problems underlying the present invention is solved in a tenth aspect by a method for intratumorally administering a virus comprising the step of administering the virus intratumorally into a tumor, wherein the virus is a virus according to any of the first, second or third aspect or any embodiment thereof.

In an embodiment of the ninth and the tenth aspect the virus is administered to or into a hypoxic area of the tumor.

In an embodiment of the first, second, third, ninth, and tenth aspect the tumor comprises cells expressing YB-1 and/or CD44.

In an embodiment of the first, second, third, ninth, and tenth aspect the tumor is a solid tumor and preferably the tumor is selected from the group comprising lymphoma, glioma, hepatocellular carcinoma, breast cancer, lung cancer, ovarian cancer, synovial sarcoma, melanoma, prostate cancer, colorectal cancer, head and neck cancer, pancreatic cancer and urothelial carcinoma.

It will be acknowledged that any embodiment described herein may be an embodiment of each and any other embodiment described herein, in particular within the individual aspects and groups of aspects.

The present inventor has surprisingly found that a particular group of adenoviruses is able to replicate under hypoxic conditions and that such replication is preferably happening to such an extent that tumor cells and tumor stem cells can be killed, preferably by oncolysis. By the adenovirus mediated killing of the tumor cells and tumor stem cells, an efficient treatment of the tumor and thus cancer is possible. The particular group of adenoviruses are those which
are YB-1 dependent adenoviruses, i.e. adenoviruses which require YB-1 for replication. In a preferred embodiment the particular group of adenoviruses is further characterized by their replication-deficiency in normal cells and/or in normal cells which have been exposed to hypoxia. Although normal cells could survive hypoxia they will not be able to support YB-1 dependent replication, since over-expression of YB-1 is only observed in transformed cells and is not induced by hypoxia. In a preferred embodiment of the instant invention, normal cells are non-tumor cells, cells which are not transformed, cells which do not show an aberrant growth characteristic, and/or cells which are not derived from a tumor or malignancies.

The above finding is insofar surprising as the understanding in the art prior to the filing of the instant application was such that the replication of adenoviruses is decreased under hypoxic conditions and that in particular adenoviruses different from wild type are therefore not suitable for being used for the killing or oncolysis of cells which are exposed to hypoxic conditions (Shen BH and Hermiston TW, Gene Therapy (2005) 12, 902-910; Pipiya T et al. Gene Therapy (2005) 12, 911-917). Said particular adenoviruses are YB-1 dependent, i.e. due to their replication being YB-1 dependent, and, compared to wild type adenovirus, are only conditionally replicating and thus replicating in a manner which typically is associated with a decrease in virus particles produced during replication and/or amount of virus DNA produced. Because of the finding that under hypoxia wild type adenovirus replication is decreased compared to replication under normoxia, a person skilled in the art would have expected that the replication of a conditionally replicating adenovirus such as the YB-1 dependent replicating adenovirus, would be even more decreased under hypoxia. Such decrease in replication under hypoxia would have been viewed as not allowing this conditionally replicating virus to kill tumor cells and tumor stem cells, respectively.

Without wishing to be bound by any theory the present inventor currently assumes that such lack of or decrease in killing tumor cells and tumor stem cells is based on a decrease in the number of viral particles arising from the replication of the adenovirus. Furthermore, because of this, any intratumoral administration of an adenovirus which was to be replicating such as a replicating oncolytic adenovirus, was performed such as to avoid the administration of the adenovirus to hypoxic areas of the tumor. Such hypoxic conditions, however, exist in some
areas of factually any tumor, more specifically any solid tumor. In the light of the discovery underlying the present invention, however, a person skilled in the art will now take into consideration that when an adenovirus is to be administered, such adenovirus may be or is actually to be administered into a hypoxic area of such tumor.

Another consequence which immediately arises from said discovery is that because of this a means is provided with the viruses according to the present invention and their use as disclosed herein, which allows the killing of tumor stem cells, in particular \textit{in vitro} and \textit{in situ}, as tumor stem cells are predominantly found in hypoxic areas of tumors. In connection with this it is to be acknowledged that hypoxic conditions are known to prompt tumor cells to adopt the characteristics of a tumor stem cell (Li et al., Cancer Cell 15, 501 - 513, 2009; McCord et al., Mol Cancer Res. 7, 489-496, 2009). In connection therewith it has to be acknowledged that tumor stem cells exhibit YB-1 which allows that said group of YB-1 dependent adenoviruses and preferably those which are disclosed herein, are able to replicate in tumor stem cells and, respectively, to kill such tumor stem cells.

As preferably used herein the term tumor stem cells comprises tumor stem cells, tumor initiating cells and/or tumor cells with tumor stem cells characteristics. It will be acknowledged by a person skilled in the art that tumor stem cells in their diverse forms, including the forms of tumor initiating cells and tumor cells with tumor stem cells characteristics share the characteristic that they are suitable for and/or capable of initiating tumor formation and/or self-renewal. In a preferred embodiment self-renewal means that a cell is dividing in an asymmetric manner. The result of such asymmetric dividing are two cells one of which is acting as a tumor initiating cell whereas the other of said two cells is not active of or capable of initiation a tumor, but is a cell forming the tumor or being part of a tumor.

In accordance with an embodiment of the various aspects of the present invention the tumor cells and tumor stem cells, respectively, and thus the tumors which can be treated by the YB-1 dependent adenoviruses are those which exhibit YB-1. Such tumor cells and tumor stem cells, respectively, are those which contain YB-1 in the nucleus, preferably in the nucleus independent from the cell cycle, and/or those which contain deregulated YB-1. In a preferred
embodiment the deregulated YB-1 is YB-1 which is phosphorylated and/or acetylated (Knott H., Ph.D. thesis, Medizinische Fakultät der Rheinisch-Westfälischen Technischen Hochschule; June 17, 2008).

To verify or detect YB-1 and/or deregulated YB-1 such as phosphorylated YB-1, a standard immunohistochemistry approach or standard Western blotting can be performed. Briefly, for Western blotting, tissue is homogenized and protein concentrations is determined using the BCA Protein Assay kit (Pierce, Rockford, USA). Protein (30-40 μg) is electrophoresed on 10% SDS polyacrylamide gels and electrophoblotted onto Hybond-ECL nitrocellulose membrane (GE Healthcare, Munich, Germany). The membrane is incubated overnight at 4°C in TBST [50 mmol/L Tris-HCl (pH 7.6), 150 mmol/L NaCl, 0.2% Tween 20] containing 5% fat-free dry milk (AppliChem, Darmstadt, Germany) and then probed with a YB-1 specific antibody (Abeam, USA; ore F-E2G5 (Dahl et al., BMC Cancer 2009, 9, 1-17) or a rabbit antibody directed to the c-terminus (CDGKETKAADPPAEN) of YB-1 (Gluz et al., JCO 2009, 27, 6144-51). To detect phosphorylated YB-1 and thus deregulated YB-1 an antibody from Cell Signaling, USA (anti-pYB-1 si02) can be used instead (Gao et al. Mol Cancer Therapy 2009, 8, 3276-3284; Stratford et al., Breast Cancer Research 2008, 10, R99). The bands are visualized using the ECL-based immunochemistry system from Roche (Roche, Penzberg, Germany). Standard immunohistochemical staining using tissue microarray for YB-1 is described in Gluz et al. (JCO 2009, 27, 6144-51) or Dahl et al. (BMC Cancer 2009, 9, 1-17).


The tumor cells and tumor stem cells, respectively, and thus the tumors which can be addressed, i.e. killed in accordance with the present invention by the adenoviruses as described herein and/or which can be subject to the methods described herein are also those which express CD44. The rationale behind this is that YB-1 regulates the expression of CD44 through the binding of YB-1 to the CD44 promoter (To K et al. Cancer Res. 2010 Apr 1;70(7):2840-51. Epub 2010 Mar 2). Because of this, the tumors which can be addressed by the adenoviruses as described herein and/or which can be subject to the methods described herein, are also and in particular the following ones showing CD44 expression: breast cancer (To K et al. Cancer Res. 2010 Apr 1;70(7):2840-51. Epub 2010 Mar 2), lung cancer (Zhang HZ et al., Cell Mol Biol (Noisy-le-grand). 2010, 56 Suppl:OLI 350-8), head and neck cancer (Prince ME et al. Proc Natl Acad Sci U S A. 2007, 104(3):973-8), prostate cancer (Hurt EM et al. Br J Cancer. 2008 Feb 26;98(4):756-65), pancreatic cancer (Lee CJ et al. J Clin Oncol. 2008, 26(17):2806-12) and urothelial carcinoma (Hagikura M. et al. Pathol Int. 2010, 60(11):735-43).

Further tumors which can be addressed, i.e. killed in accordance with the present invention by the adenoviruses as described herein and/or which can be subject to the methods described herein are preferably those tumors which are selected from the group comprising tumors of the nervous system, ocular tumors, tumors of the skin, tumors of the soft tissue, gastrointestinal tumors, tumors of the respiratory system, tumor of the skeleton, tumors of the endocrine system, tumors of the female genital system, tumors of a mammary gland, tumors of the male genital system, tumors of the urinary outflow system, tumors of the hematopoietic system including mixed and embryonic tumors. It is within the present invention that these tumors are in particular resistant tumors as in particular defined herein.

The group of tumors of the nervous system which may be subject to the methods of the present invention preferably comprises:
1. Tumors of the skull as well as of the brain (intracranial), preferably astrocytoma, oligodendroglioma, meningioma, neuroblastoma, ganglieneuroma, ependymoma, schwannoglioma, neurofibroma, haemangioblastoma, lipoma, craniopharyngioma, teratoma and chordoma;

2. Tumors of the spinal cord and of the vertebral canal, preferably glioblastoma, meningioma, neuroblastoma, neurofibroma, osteosarcoma, chondrosarcoma, haemangiosarcoma, fibrosarcoma and multiple myeloma; and

3. Tumors of the peripheral nerves, preferably schwannoglioma, neurofibroma, neurofibrosarcoma and perineural fibroblastoma.

The group of the ocular tumors which may be subject to the methods of the present invention preferably comprises:

1. Tumors of the eyelids and of the lid glands, preferably adenoma, adenocarcinoma, papilloma, histiocytoma, mast cell tumor, basal-cell tumor, melanoma, squamous-cell carcinoma, fibroma and fibrosarcoma;

2. Tumors of the conjunctiva and of the nictitating membrane, preferably squamous-cell carcinoma, haemangioma, haemangiosarcoma, adenoma, adenocarcinoma, fibrosarcoma, melanoma and papilloma; and

3. Tumors of the orbita, the optic nerve and of the eyeball, preferably retinoblastoma, osteosarcoma, mast cell tumor, meningioma, reticular cell tumor, glioma, schwannoglioma, chondroma, adenocarcinoma, squamous-cell carcinoma, plasma cell tumor, lymphoma, rhabdomyosarcoma and melanoma.

The group of skin tumors which may be subject to the methods of the present invention preferably comprises:
Tumors of the histiocytoma, lipoma, fibrosarcoma, fibroma, mast cell tumor, malignant melanoma, papilloma, basal-cell tumor, keratoacanthoma, haemangiopericytoma, tumors of the hair follicles, tumors of the sweat glands, tumors of the sebaceous glands, haemangioma, haemangiosarcoma, lipoma, liposarcoma, malignant fibrous histiocytoma, plasmacytoma and lymphangioma.

The group of tumors of the soft-tissues which may be subject to the methods of the present invention preferably comprises:

Tumors of the alveolar soft-tissue sarcoma, epithelioid cell sarcoma, chondrosarcoma of the soft-tissue, osteosarcoma of the soft-tissues, Ewing's sarcoma of the soft-tissues, primitive neuroectodermal tumors (PNET), fibrosarcoma, fibroma, leiomyosarcoma, leimyoma, liposarcoma, malignant fibrous histiocytoma, malignant haemangiopericytoma, haemangioma, haemangiosarcoma, malignant mesenchymoma, malignant peripheral nerve sheath tumor (MPNST, malignant schwannoglioma, malignant melanocyte schwannoglioma, rhabdomyosarcoma, synovial sarcoma, lymphangioma and lymphangiosarcoma.

The group of gastrointestinal tumors which may be subject to the methods of the present invention preferably comprises:

1. Tumors of the oral cavity and of the tongue, preferably squamous-cell carcinoma, fibrosarcoma, Merkel cell tumor, inductive fibroameloblastoma, fibroma, fibrosarcoma, viral papillomatosis, idiopathic papillomatosis, nasopharyngeal polyps, leiomyosarcoma, myoblastoma and mast cell tumor;

2. Tumors of the salivary glands, preferably adenocarcinoma;

3. Tumors of the oesophagus, preferably squamous-cell carcinoma, leiomyosarcoma, fibrosarcoma, osteosarcoma, Barrett carcinoma and paraoesophageal tumors;

4. Tumors of the exocrine pancreas, preferably adenocarcinoma; and
5. Tumors of the stomach, preferably adenocarcinoma, leiomyoma, leiomyosarcoma and fibrosarcoma.

The group of the tumors of the respiratory system which may be subject to the methods of the present invention preferably comprises:

1. Tumors of the nose and nasal cavity, of the larynx and of the trachea, preferably squamous-cell carcinoma, fibrosarcoma, fibroma, lymphosarcoma, lymphoma, haemangioma, haemangiosarcoma, melanoma, mast cell tumor, osteosarcoma, chondrosarcoma, oncocytoma (rhabdomyoma), adenocarcinoma and myoblastoma; and


The group of the skeleton tumors which may be subject to the methods of the present invention preferably comprises:

osteosarcoma, chondrosarcoma, parosteal osteosarcoma, haemangiosarcoma, synovial cell sarcoma, haemangiosarcoma, fibrosarcoma, malignant mesenchymoma, giant-cell tumor, osteoma and multilobular osteoma.

The group of the tumors of the endocrine system which may be subject to the methods of the present invention preferably comprises:

1. Tumors of the thyroid gland/parathyroid, preferably adenoma and adenocarcinoma;
2. Tumors of the suprarenal gland, preferably adenoma, adenocarcinoma and pheochromocytoma (medullosuprarenoma);

3. Tumors of the hypothalamus/hypophysis, preferably adenoma and adenocarcinoma;

4. Tumors of the endocrine pancreas, preferably insulinoma (beta cell tumor, APUDom) and Zollinger-Ellison syndrome (gastrin secreting tumor of the delta cells of the pancreas); and

5. as well as multiple endocrine neoplasias (MEN) and chemodectoma.

The group of the tumors of the female sexual system tumors which may be subject to the methods of the present invention preferably comprises:

1. Tumors of the ovaries, preferably adenoma, adenocarcinoma, cystadenoma, and undifferentiated carcinoma;

2. Tumors of the uterine, preferably leiomyoma, leiomyosarcoma, adenoma, adenocarcinoma, fibroma, fibrosarcoma and lipoma;

3. Tumors of the cervix, preferably adenocarcinoma, adenoma, leiomyosarcoma and leiomyoma;

4. Tumors of the vagina and vulva, preferably leiomyoma, leiomyosarcoma, fibroleiomyoma, fibroma, fibrosarcoma, polyps and squamous-cell carcinoma.

The group of tumors of the mammary glands which may be subject to the methods of the present invention preferably comprises:

fibroadenoma, adenoma, adenocarcinoma, mesenchymal tumor, carcinoma, carcinosarcoma.
The group of the tumors of the male sexual system which may be subject to the methods of the present invention preferably comprises:

1. Tumors of the testicles, preferably seminoma, interstitial-cell tumor and Sertoli cell tumor;

2. Tumors of the prostate, preferably adenocarcinoma, undifferentiated carcinoma, squamous-cell carcinoma, leiomyosarcoma and transitional cell carcinoma; and

3. Tumors of the penis and the external gentals, preferably mast cell tumor and squamous-cell carcinoma.

The group of tumors of the urinary outflow system which may be subject to the methods of the present invention preferably comprises:

1. Tumors of the kidney, preferably adenocarcinoma, transitional cell carcinoma (epithelial tumors), fibrosarcoma, chondrosarcoma (mesenchymal tumors), WilnVs tumor, nephroblastoma and embryonal nephroma (embryonal pluripotent blastoma);

2. Tumors of the ureter, preferably leiomyoma, leiomyosarcoma, fibropapilloma, transitional cell carcinoma;

3. Tumors of the urinary bladder, preferably transitional cell carcinoma, squamous-cell carcinoma, adenocarcinoma, botryoid (embryonal rhabdomyosarcoma), fibroma, fibrosarcoma, leiomyoma, leiomyosarcoma, papilloma and haemangiosarcoma; and

4. Tumors of the urethra, preferably transitional cell carcinoma, squamous-cell carcinoma and leiomyosarcoma.

The group of tumors of the haematopoietic system which may be subject to the methods of the present invention preferably comprises:

The group of the mixed and embryonal tumors which may be subject to the methods of the present invention preferably comprises:

Haemangiosarcoma, thymoma and mesothelioma.

Further tumors are those which are resistant as described herein, preferably these which are multiple resistant, particularly also those tumors of the group described above.

Methods for determining hypoxic regions in a tumor and thus for the administration of an agent and thus of the viruses according to the present invention into such tumor, are known to the person skilled in the art and, for example, described in Spence AM et al. (Clin Cancer Res 2008; 14(9) May 1, 2008. More specifically, such hypoxic regions can be determined by [18F]Fluoromisonidazole Positron Emission Tomography (PET) (Spence AM, Clin Cancer Res 2008;14(9)May 1, 2008), by the use of 18F-Fluoroazomycin Arabinoside (Piert M, THE JOURNAL OF NUCLEAR MEDICINE, Vol. 46, No. 1, January 2005) or pulsed electron paramagnetic resonance imaging (EPRI) with conventional MRI (Manzoor AA, The Journal of Clinical Investigation, volume 118, number 5, May 2008).

In an embodiment of the various aspects of the invention disclosed herein, it is understood that hypoxia exists in vivo at 10 mm Hg, whereas 50 mmHg correspond to normoxia. In an embodiment of the various aspects of the invention disclosed herein, 0.66 % O_2 correspond to about 5 mm Hg (Schilling D., The FASEB Journal, Vol. 23 August 2009, pp 2467 - 2477). It is also generally accepted in the art that the percentage of oxygen used or present in in vitro studies referring to hypoxia or hypoxic conditions, are the same as or essentially correspond to the percentage of oxygen used or present in in vivo studies referring to hypoxia or hypoxic conditions. The same applies to normoxia and normoxic conditions.
YB-1 dependent adenoviruses which can be used in accordance with the present invention are, among others, described in international patent applications WO 03/099859 and WO 2004/035616 and are described herein in more detail. A preferred embodiment of such YB-1 dependent adenovirus is dl520. dl520 is a recombinant adenovirus which differs from type 5 adenovirus of the wild type insofar that from the two forms of the E1A protein, i.e. E1A12S and E1A13S only the E1A12S protein is actually expressed and therefore available so as to exercise its activity. The presence of E1ADS in the adenoviral genome is insofar important since this protein is required to activate viral genes from the E2-, E3- and E4.region (Berk A., Oncogene 2005, 24, 7673-85). To test for the presence of E1A13S in an adenovirus vector a standard real-time PCR protocol is available (El Hassan et al., Journal of Virology 2006, 133, 53-61). In a particularly preferred embodiment dl520 is dl520 which expresses RGD (which is a trimer of aminoacids arginine, glycine and aspartate).

The viruses used in accordance with the present invention, in particular adenoviruses, are viruses, which are YB-1 dependent, i.e. require YB-1 for replication. In connection therewith it is within the present invention that such viruses are already known in the prior art and can be used in accordance with the present invention accordingly, or that such virus can be designed based on the disclosure provided herein. It is to be noted that the term viruses or adenoviruses in accordance with the invention or viruses or adenoviruses used in accordance with the invention, is insofar irrelevant for the purposes of the present invention that the viruses described herein may be used in accordance with the present invention provided that they use YB-1 for replicating. The YB-1 used for replication can be YB-1 which is either deregulated, preferably as defined herein, or localized in the nucleus, in particular localized in the nucleus independent of the cell cycle.

Cells which contain YB-1 in the deregulated form, are those which comprise at least one of the following characteristics and/or those which contain YB-1, whereby the YB-1 exhibits at least one of the following characteristics: (1) YB-1 is overexpressed in the cells, preferably independent of the cell cycle, whereby, preferably, as a measure for expression the expression of YB-1 in normal cells is used, i.e. cells which are different from tumour cells or cells and cell lines, respectively, such as the followings: Hepatocytes as well as fibroblast cell lines WI38 and CCD32-Lu. Preferably, there is an overexpression when the expression is increased
by a factor ranging from 2 to 10, preferably from 5 to 10. Methods for measuring the expression and in particular measuring the overexpression are known to the one skilled in the art and comprise, among others, measuring the protein concentration, in particular the protein concentration of YB-1, measuring RNA, in particular of YB-1, Western Blot analysis, Northern Blot analysis and RT-PCR, each preferably of or in relation to YB-1. Rather than YB-1, also surrogate markers can be used as described herein. Examples for cell lines which show an overexpression of YB-1, are the followings: colon carcinoma cell line 257RDB, pancreas carcinoma cell line 181RDB, mamma carcinoma cell line MCF-7Adr, prostate carcinoma cell line DU145, prostate carcinoma cell line PC3, glioma cell line U373, glioma cell line U87, lung carcinoma cell line A549, liver carcinoma cell lines Hep3B and HepG2. (2) The YB-1 present in the cell enables the replication of the viruses in accordance with the present invention. In connection with the present invention it is preferred, when the replication efficiency under such conditions is different from a replication which is significantly reduced.

In an embodiment a significantly reduced replication is in particular a replication which is, compared to wild type, reduced by a factor of 2, preferably by a factor of 5, more preferably by a factor of 10 and most preferably by a factor of 100. In a preferred embodiment comparing the replication is done by using similar or identical cell lines, similar or identical virus titres for infection (in English multiplicity of infection, MOI, or, in English, plaque forming unit, pfu) and/or identical or similar general experimental conditions. The term replication in particular refers to particle formation. In a further embodiment the extent of viral nucleic acid synthesis can be understood as measure for replication. Methods for determining the extent of viral nucleic acid synthesis are known to the one skilled in the art as well as methods for determining particle formation.

The diseases and patients, respectively, to be treated are those as described herein. With regard to the timing between the administration of the viruses in accordance with the present invention and radiation or administration of the cytostatics it is to be noted that it is predominantly determined by the replication efficiency of the viruses and the kind and size of the tumour. It will be appreciated by the one skilled in the art that, upon administration of the viruses, it may last about one to three days, until the replication and thus the complexing of
YB-1 and, accordingly, its non-availability for the transcription of other factors and of respective resistance causing factors in particular, occurs. Insofar in particular at the beginning of the treatment of resistant diseases the administration of viruses prior to any further treatment, in particular administration of pharmaceutical active compounds and/or radiation, is advantageous.

The administration of a pharmaceutically active compound preferably comprises the administration of an anti-tumour or anti-cancer agent as disclosed herein by way of example. Further respective agents are known to the one skilled in the art. Particularly preferred are cytostatics. Exemplary cytostatics are those described herein in connection with the pharmaceutical compositions and the (pharmaceutical) agent which is administered together with the virus.

It is within the present invention that the dosages and treatments schemes as customary in the treatment of tumour diseases may also be applied in connection with the present invention. For example, the amount of the cytostatic to be administered is preferably calculated based on the body surface (as m\(^2\)); by means of example, the dosage of Doxorubicin is 50 mg/m\(^2\). The therapeutical schemes can be designed differently and comprise single day administration as well as administration of the cytostatic and radiation, respectively, for several days. The administration and radiation may additionally occur in a cyclic manner. Radiation and/or administration of the cytostatic may in turn be a mono- or combination therapy which, in accordance with the present invention, is further complemented by the administration of the virus.

In the following, various adenoviruses are described which may be used as viruses in accordance with the present invention. In connection therewith, only for reasons of clarity they are categorized into groups and referred to as virus #1, virus #2 and virus #3. The adenovirus which can be used in accordance with the present invention are in particular also those adenoviruses which are described in international patent applications WO 03/099859 and WO 2004/035616. It is within the present invention that the comments provided in relation to the individual groups are applicable to the other groups as well, provided that this
is not explicitly excluded; this applies in particular to the definitions provided in the specific embodiments.

As used herein in an embodiment, the term functional wild type E1 region refers in particular to an E1 region as contained in the adenovirus Ad5 of the wild type. In an embodiment the term lacking functional wild type E1 region refers to an E1 region which either does not comprise one or several functions or functionalities of the E1 region as present in wild type adenoviruses or which does not completely comprise the same. The functionality or function, in the following generally referred to as function, is mediated by a nucleic acid or a protein, preferably is represented or mediated by a protein.

In connection with the present invention the lack of function can be caused by the function not being active at the level of translation, i.e. that the function mediating protein is not present although the nucleic acid coding therefore is still present in the viral genome. This can, for example, be caused by the regulatory elements controlling its translation being absent, such as, for example, the 3'UTR of the mRNA which, among others, provide for the stability of the mRNA. Preferably these regulatory elements are no longer present in the regulatory and controlling context as present in wild type viruses for the respective function.

In connection with the present invention the lack of function can, alternatively or additionally, be caused by the function not being active at the level of transcription, i.e. that the protein mediating the function is not present and the nucleic acid coding therefore, is not contained in the viral genome or not completely contained in the viral genome. It is within this embodiment that the coding nucleic acid comprises one or several mutations which result in the loss of function. Such mutations are preferably point mutations and/or deletions comprising several bases and/or a complete deletion of the open reading frame or the nucleic acid coding for the protein.

A function is lacking in the sense of the above embodiments if the protein does not comprise all functions or activities as exhibited by the corresponding wild type protein. In an embodiment, the extent of replication is used as a measure for activity which can be obtained
under such conditions, whereby it is preferably different from a significantly different replication.

In a preferred embodiment of the present invention there is a lack of function also when the function is, compared to the wild type virus, contained in the virus in a different regulatory context. A different regulatory context is in a preferred embodiment a context in connection with which the function is, compared to other functions, expressed at a different point in time and/or is under the control of a different transcription and/or translation controlling or influencing element. Such an element is in a particular embodiment the promoter.

In an embodiment a strongly reduced replication herein in particular means a replication which is decreased compared to the wild type by a factor of 2, preferably a factor of 5, more preferably a factor of 10 and most preferably a factor of 100. In a preferred embodiment the comparison of the replication is made using identical or similar cell lines, identical or similar virus titres for the infection (multiplicity of infection, MOI or plaque forming unit, pfu) and/or identical or similar general experimental conditions. Replication particularly means the formation of particles. In further embodiments the measure for replication may be the extent of viral nucleic acid synthesis. Methods for determining the extent of viral nucleic acid synthesis and methods for the determining particle formation are both known to the ones skilled in the art.

The lack of a function in the above sense is herein also indicated by the respective function being referred to as "minus". For example, the lack of E1A13S is indicated as E1A13S-minus.

Virus group #1

This group of viruses is based on the surprising finding that the DNA replication of E1A-modified adenoviruses in YB-1 nucleus positive tumour cells is based on the activation of the E2-late promoter. E1A-modified adenoviruses as used herein, are adenoviruses which (a) do not replicate in YB-1 nucleus-negative cells or show a reduced, preferably a strongly reduced replication in YB-1 nucleus-negative cells compared to the respective wild type, (b)
transactivate at least one viral gene, whereby the gene is in particular selected from the group comprising ElB-55kDa, E4orf6, E4orf3 and E3ADP, and/or (c) do not translocate cellular YB-1 through the adenovirus into the nucleus. Optionally the adenoviruses used in accordance with the present invention have the further characteristic that the binding of the adenoviral encoded E1A protein interferes with the binding of E2F to Rb and is able to dissolve the respective complex consisting of E2F and Rb, respectively. Adenoviruses which have at least one or several of the aforementioned features a) to c), preferably all of features a) to c), are replication deficient in cells which do not have YB-1 in the nucleus.

Without wishing to be bound by this in the following, the present inventor assumes that the E2-early promoter, i.e. the early E2 promoter is not switched on through the human cellular E2F transcription factor in connection with the replication of the viruses used herein in accordance with the present invention. The switching on of the replication is independent of the Rb status of the cells, i.e. which means that the tumor cells which are infected using the viruses disclosed herein and which are preferably lysed subsequently thereafter, may comprise both functional as well as inactive Rb proteins. Additionally, adenoviral replication does neither need any functional p53 protein nor is it affected by its presence, when using the adenoviruses disclosed herein or under the conditions disclosed herein. Insofar, the technical teaching departs from the principle underlying the use of the oncolytic or tumorlytic adenoviruses of the AdA24, d1922-947, ElAd/01/07, CB016 type or of those adenoviruses which are, for example, described in European patent EP 0 931 830, and into which one or several deletions have been introduced into the E1A protein under the assumption that intact functional Rb proteins are an obstacle to an efficient replication in vivo thus providing an adenoviral replication in vivo only in Rb-negative and Rb-mutated cells, respectively. These adenoviral systems according to the prior art are based on E1A in order to control in vivo replication of adenoviruses by means of the early E2 promoter (E2 early promoter) and "free E2F". Nevertheless, these viruses according to the prior art may be used in accordance with the present invention, i.e. for replication in cells which contain YB-1 in the nucleus independent from the cell cycle.

The viruses described in said European patent EP 0 931 830 and in particular adenoviruses may be used in accordance with the present invention. More particularly, the viruses
described in said patent are replication deficient and lack an expressed viral oncoprotein which is capable of binding a functional Rb tumor suppressor gene product. The adenovirus can particularly be an adenovirus which is lacking expressed viral E1A oncoprotein which is capable of binding a functional tumor suppressor gene product, in particular Rb. The viral E1A oncoprotein can comprise an inactivating mutation, for example in the CR1 domain at amino acid positions 30 to 85 in Ad 5, nucleotide positions 697 to 790 and/or the CR2 domain at amino acid positions 120 to 139 in Ad 5, nucleotide positions 920 to 967 which are involved in the binding of p105 Rb protein, p130 and p107 protein. It can also be intended that the adenovirus is of type 2 dl 312 or the adenovirus is of type 5 NT dl 1010.

A further feature of the adenoviruses which are to be used in accordance with the invention, is that they code for a viral oncoprotein which is also referred to herein as oncogene protein, whereby the oncogene protein is preferably E1A, whereby the oncogene protein is capable of activating at least one viral gene which can have an impact on the replication of the virus and/or cell lysis of the cells infected by the virus. It is preferred that the influence on replication is such that the virus replicates better in the presence of the oncogene protein compared to a situation where the oncogene protein of the respective virus is lacking. This process is referred to herein also as transactivating and in particular E1A transactivating, when the transactivation is mediated through E1A. The term "transactivate" or "transactivation" describes preferably the process that the respective viral oncoprotein has an impact on the expression and/or the transcription of one or several other genes different from the viral oncoprotein coding gene itself, i.e. is preferably controlling its expression and/or translation, and in particular activates this/these. Such viral genes are preferably ElB55kDa, E4orf6, E4orf3 and E3ADP as well as any combination of the aforementioned genes and gene products, respectively.

A further, although preferably optional, feature of the adenoviruses to be used in accordance with the invention, is the binding to and of tumor suppressor Rb. In principle it is within the present invention that the adenoviruses used in accordance with the present invention bind to Rb or do not bind to Rb. The use of both alternative embodiments of the adenoviruses is possible independently from the Rb status of the cell to be treated.
In order to confer the capability to not bind to Rb, the following deletions of the E1A oncoprotein are, for example, possible: Deletion in the CR1 region (amino acid positions 30 - 85 in Ad5) and deletion of the CR2 region (amino acid positions 120 - 139 in AD5). In doing so, the CR3 region is maintained and can have its transactivating function on the other early viral genes.

In contrast thereto, the following deletions to the E1A oncoprotein are in principle possible in order to impart E1A the capability to bind to Rb: deletion of the CR3 region (amino acid positions 140 - 185); deletion of the N-terminus (amino acid positions 1 - 29); deletion of amino acid positions 85 - 119; and deletion of the C-terminus (amino acid positions 186 - 289). The regions recited herein do not interfere with the binding of E2F to Rb. The transactivating function remains, however, is reduced compared to wild type Ad5.

In connection with the present invention the modified E1A oncoprotein of the various adenoviruses which are to be used in accordance with the invention, is capable of transactivating the early viral genes such as, for example, E1B55K, E4orf3, E4orf6, E3ADP, in YB-1 nucleus-positive cells. In connection therewith, there are preferably otherwise no further changes to the viral genome and the respective adenovirus can otherwise correspond to an adenovirus of the wild type or any derivative thereof.

The viruses disclosed herein which code for a transactivating oncogene protein in the sense of the present invention or which comprise such oncogene protein, comprise, for example, the adenoviruses AdA24, d1922-947, E1Ad/01/07, CB106 and/or the adenoviruses described in European patent EP 0 931 380, which are each capable of transactivating the early genes, such as E1B, E2, E3 and/or E4, and are comparable to adenoviruses of the wild type, in particular wild type Ad5. A particular region of the E1A protein is responsible for transactivation in these cases. Within various adenovirus serotypes there are three highly conserved regions in the E1A protein. The CR1 region from amino acid positions 41 - 80, the CR2 region from amino acid positions 120 - 139 and the CR3 region from of amino acid positions 140 - 188. The transactivating function is primarily based on the presence of the CR3 region in the E1A protein. The amino acid sequence of CR3 is unaltered in the aforementioned adenoviruses.
This results in a transactivation of the early genes E1B, E2, E3 and E4 independent from the presence of YB-1 in the nucleus or in the cytoplasm.

In the recombinant adenovirus dl520, however, the CR3 region has been deleted. Thus dl520 expresses a so-called E1A12S protein which does not comprise the amino acid sequence of the CR3 region. As a consequence, dl520 can exert a very weak transactivating function only, in particular on the E2 region, and thus does not replicate in YB-1 nucleus-negative cells. In YB-1 nucleus-positive cells YB-1 is transactivating the E2 region and thus allows an efficient replication of dl520. This is the basis for the use of systems like dl520 and of systems on the basis of dl520 for the purposes disclosed herein, respectively. A further important difference between both the previously described groups of adenoviruses, i.e. delta 24 (herein also referred to as AdA24) and dl520 resides in the fact that with dl520 the early genes E1B, E3 and E4 are more strongly transactivated in YB-1 nucleus-positive cells compared to YB-1 nucleus-negative cells. In contrast, there are no or only minor differences with delta 24. The transactivation effect of dl520 and more particularly of the E1A12S protein, however, is significantly reduced compared to wild type adenovirus. This transactivation is, however, sufficient in order to allow for an efficient replication in YB-1 nucleus-positive cells. The design of the E1A protein and of the nucleic acid coding therefor described herein and in particular in this context such that the E1A protein has one or several deletions and/or mutations compared to the wild type oncogene protein E1A, whereby the deletion is preferably one selected from the group comprising deletions of the CR3 region and deletions of the N-terminus and deletions of the C-terminus, including and particularly preferred those embodiments of the E1A protein as described in connection with dl520 or AdA24, dl922-947, E1Ad/01/07, CB106 and/or the adenoviruses described in European patent EP 0 931 830, are embodiments of viruses, in particular adenoviruses, the replication of which is controlled by YB-1 through the activation of the E2-late promoter, preferably predominantly through the activation of the E2-late promoter. Further embodiments of the E1A protein which allow this form of replication of adenoviruses, can be generated by the ones skilled in the art based on the disclosure provided herein.

In further adenoviruses which are to be newly constructed, which are also referred to herein as derivatives and which may be used in accordance with the present invention, typically have
an E1 deletion, an E1/E3 deletion and/or an E4 deletion, i.e. the corresponding adenoviruses are not able to generate functionally active E1 and/or E3 and/or E4 expression products and respective products, respectively, or, in other words, these adenoviruses are only capable to generate functional inactive El, E3 and/or E4 expression products, whereby a functionally inactive El, E3 and/or E4 expression product as such which is either not present as an expression product at all, whether at the transcription level and/or the translation level, or it is present in a form in which it at least is lacking one of the functions it has in wild type adenoviruses. The function(s) of the expression product of the wild type adenovirus is/are known to the ones skilled in the art and, for example, described in Russell, W. C., Journal of Virology, 81, 2573-2604, 2000. Russell (supra) describes also principles for the construction of adenoviruses and adenoviral vectors which are incorporated herein by reference. It is also within the present invention that the modified E1A oncoprotein, E1B-55K, E4orf6 and/or E3ADP (adenoviral death protein (ADP)) (Tollefson, A. et al., J. Virology, 70, 2296-2306, 1996) is expressed in such a vector either individually or in any combination. In connection therewith, the individually named genes as well as the transgenes disclosed herein, can be cloned into the E1 and/or E3 and/or E4 region and be expressed independently by virtue of a suitable promoter or under the control of a suitable promoter. Basically, the regions El, E3 and E4 are similarly suitable as cloning sites within the adenoviral nucleic acid, whereby the regions which are not used for the cloning may, either individually or all together, be present, partially deleted and/or completely deleted. In case these regions are present, in particular are completely present, it is within the present invention that they are either intact and preferably provide for a translation product and/or a transcription product, and/or are not intact and preferably do not provide for a translation product and/or a transcription product. Suitable promoters are, for example those, which are disclosed herein in connection with the control and expression, respectively, of E1A, in particular of modified E1A.

Finally, in one embodiment the adenoviruses which are to be used in accordance with the present invention, are deficient with regard to E1B, in particular with regard to E1B 19 kDa. As used herein, the term deficient generally means a condition in which E1B does not have all of the characteristics inherent to the wild type but at least one of these characteristics is absent. The adenoviral BCL2 homologue E1B19k inhibits the E1A induced apoptosis by interacting with the pro-apoptotic proteins Bak and Bax. Because of this, a maximum
replication and/or particle formation is possible in infected cells (Ramya Sundararajan und Eileen White, Journal of Virology 2001, 75, 7506-7516). The lack of E1B19k results in a better release of the viruses as it minimizes the function of the adenoviral death-protein, if present. The virus induced cytophatic effect is increased by such deletion (Ta-Chiang Liu et al., Molecular Therapy, 2004) and thus results in a stronger lysis of the infected tumour cells. Additionally, the lack of E1B19k results in TNF-alpha not having an impact on the replication of such recombinant adenovirus in tumour cells, whereas in normal cells the treatment results in a reduced replication and release of infectious viruses. Thus the selectivity and specificity is increased (Ta-Chiang Liu et al., Molecular Therapy 2004, 9, 786-803).

The adenoviruses which are used in accordance with the invention disclosed herein, are, basically, known in the prior art in some embodiments. The adenoviruses used in accordance with the present invention are preferably recombinant adenoviruses, particularly also when a change, compared to the wild type, has been made in accordance with the technical teaching provided herein. It is within the skills of those of the art to delete or mutate those adenoviral nucleic acid sequences which are not essential for the present invention. Such deletions may, for example, be related to a part of the nucleic acid coding for E3 and E4 as also described herein. A deletion of E4 is particularly preferred if such deletion does not extend to the protein E4orf6, or, in other words, the adenovirus to be used in accordance with the present invention codes for E4orf6. In preferred embodiments these adenoviral nucleic acids may still be packed into the viral capsid and may thus form infectious particles. The same is true for the use of the nucleic acids in accordance with the present invention. It should be noted that in general the adenoviral systems may be deficient with regard to single or several expression products. In connection therewith it is to be taken into consideration that this may be either based on the fact that the nucleic acid coding for such expression product is completely mutated or deleted or mutated or deleted to the extent that essentially no expression product is produced anymore or based on the lack of promoters or transcription factors which control the expression, or which are active in a manner different from wild type, either at the nucleic acid level (lack of a promoter; cis-acting element) or at the translation system and the transcription system, respectively (trans-acting elements). Particularly the latter aspect may be dependent on the cellular background.
Apart from using adenoviruses in accordance with the present invention, which are already known, also novel adenoviruses can be used to the same extent as has already been disclosed for the other adenoviruses described herein. The novel adenoviruses according to the invention result from the technical teaching provided herein. Particularly preferred representatives are, for example, the viruses Xvir03 and Xvir03/01.

In the case of vector Xvir03 a CMV promoter is cloned into the E1 region which codes the nucleic acids for E1B 55K and E4orf6, which are separated by a IRES sequence. In connection therewith the E3 region can be partially or completely be deleted or can be present and intact. Due to the introduction of these two genes and the gene products produced therefrom, respectively, a replication efficiency is created which nearly corresponds to the one of wild type viruses, whereby the selectivity of the replication is maintained for cells, particularly tumor cells, insofar as a replication happens in particular in YB-1 nucleus-positive cells and more particularly in cells in which YB-1 is deregulated. Cells in which YB-1 is deregulated, are preferably those which show an increased expression of YB-1, preferably compartment-independent, compared to normal or non-tumor cells. The introduction of E1B55k and E4orf6 into the E4-region by cloning can also be performed, whereby the E3 region may be intact or/and partially or completely deleted.

A further development of virus Xvir03 is virus Xvir03/01 into which, in a preferred embodiment, therapeutic genes or transgenes are cloned under the control of a specific promoter, in particular a tumor-specific or tissue-specific promoter. It is also within the scope of such a virus that also the E4 region is functionally inactive, preferably is deleted. The transgenes described herein may also be cloned into the E4 region, whereby this may occur in addition or alternative to the cloning of a transgene into the E3 region, and the E3-region remains partially or completely intact. Transgenes, as used herein, may be therapeutic genes or viral genes, preferably adenoviral genes, which are preferably not present in the genome of the wild type adenovirus or at the position in the genome, respectively, where they are present in the particular virus now.

In a preferred embodiment, with regard to the adenoviruses according to the present invention and the adenoviral replication system according to the present invention and the use of them
according to the present invention, respectively, the adenoviral nucleic acid is deficient for the expression of the oncogene protein, particularly of the E1A protein, which means that it is either not coding for the 12S E1A protein or for the 13S E1A protein, or it is neither coding for the 12S E1A protein nor the 13S E1A protein, or is modified, as defined herein, and that the adenoviral replication system further comprises a nucleic acid of a helper virus, whereby the nucleic acid of the helper virus comprises a nucleic acid sequence which codes for the oncogene protein, in particular for the E1A protein, which has the following characteristics and imparts the following characteristics to the adenovirus, respectively, namely that it preferably is not replicating in YB-1 nucleus-negative cells but in cells which are independent from the cell cycle YB-1 nucleus-positive, transactivating at least one viral gene, in particular E1B55kDa, E4orf6, E4orf3 and/or E3ADP, in YB-1 nucleus-positive cells, and/or does not translocate cellular YB-1 into the nucleus. It is within the present invention that the transgenes described herein are coded individually or together by the helper virus and/or expressed therefrom.

Virus group #2

These viruses are categorized into group I and group II. The viruses first defined in the claims related to virus group #2 as independent viruses, are also referred to herein as adenovirus of group I, and the adenoviruses which comprise a transactivating oncogene protein such as E1A and/or those which are referred to herein and in particular above as to be used in accordance with the present invention, are also referred to herein as adenovirus of group II. Adenovirus of group I and group II are all together also referred to herein as adenoviruses or adenoviruses in accordance with the invention or viruses in accordance with the present invention.

These viruses are based on the surprising finding that reversing the expression sequence of adenoviral genes results in an efficient replication and optionally in the lysis of the cell infected by the adenovirus. With regard to the chronologically changed expression of the adenoviral genes particular emphasis is to be put on an E1B protein and an E4 protein which are also referred to herein, individually or collectively, as the first protein, which are expressed prior to a second protein. The second protein is selected from the group comprising E1A proteins. This expression sequence which is reversed compared to wild type
adenoviruses where first an E1A protein and only subsequently the E1B protein and an E4 protein are expressed, ensures that transcription factors are activated, for example transported, into the nucleus of the infected cell and influence the further replication activity or control the same there. The kinetics of the adeno-viral transcripts in wild type adenoviruses are, for example, described in Glenn G. M. and Ricciardi R. P. Virus Research 1988, 9, 73-91, who report that in the wild type the E1A transcripts, i.e. the E1A12S transcript and the E1A13S transcript, are usually detectable prior to the transcripts and translation products, respectively, E4orf6 and ElB55k. In the present case the E1B protein is, and also herein in general if not indicated to the contrary, preferably the ElB-55kD protein. In the present case, the E4 protein is, and also herein in general if not indicated to the contrary, preferably the E4orf6 protein. In the present case, the E1A protein is, and also herein in general if not indicated to the contrary, preferably an E1A12S protein or such an E1A protein as described herein in connection with the E1A-modified adenoviruses.

It is within these viruses that the E1A protein, in particular also the E1A12S protein may be substituted in principle. Such substituted E1A proteins and E1A12S proteins, respectively, are also referred to herein as E1A protein and E1A12S protein, respectively, or shall be deemed to be comprised by this term, if not indicated to the contrary. Instead of the E1A12S protein also an E1A protein may be used which has a tumor suppressor function, such as, for example, described by Dickopp A, Esche H, Swart G, Seeber S, Kirch HC, Opalka B. Cancer Gene Ther. 2000, Jul;7(7): 1043-50. Further derivatives of E1A proteins, in particular of the E1A12S protein, as used and/or as referred to as such herein, are generally also such proteins which are capable of releasing the factor E2F from the Rb/E2F complex. These are, among others, Simian virus 40 tumor antigen (SV40 large T antigen), papillomavirus E7 protein (HPV E7) as described by Chellappan S. et al., Proc. Natl. Acad. Sci. USA 1992, 89, 4549-4553.

It is also within these viruses that derivatives of E4orf6 and ElB55k may be used, whereby the term E4orf6 and ElB55k, as used herein, comprises such derivatives. The derivatives are, for example, described in Shen Y et al., J. of Virology 2001, 75, 4297-4307; Querido E. et al., J. of Virology 2001, 75, 699-709.
It is within these viruses that an EIB protein is expressed prior to the E1A protein, or that an E4 protein is expressed prior to an E1A protein, or that both an EIB protein and an E4 protein are expressed prior to the E1A protein, each as described above.

An adenovirus designed in such a way is capable of replicating at a particularly high level upon infection of a cell which expresses YB-1 in the nucleus, preferably expresses YB-1 in the nucleus independent from the cell cycle, or which comprises deregulated YB-1, preferably in the cytoplasm. Without wishing to be bound thereto in the following the present inventor assumes that a complex consisting of EIB protein and/or E4 protein and individual ones of these two proteins, respectively, is/are capable of transporting deregulated YB-1 into the cellular nucleus or is/are capable of initiating adenoviral replication there under the influence of the EIB protein and/or E4 protein being expressed prior to the E1A protein. Once in the cellular nucleus or being present there in activated form, YB-1 may, as described herein, in particular using the E2-late promoter, efficiently replicate. The chronologically early expression of an EIB protein and/or an E4 protein thus avoids the cascade as observed in wild type going along with initial expression of E1A protein. In a preferred embodiment the E1A protein is an E1A protein which is in particular no longer transactivating or transactivating only to a very limited extent the EIB protein and/or the E4 protein. Preferably, this transactivation is neither sufficient to ensure an efficient replication, nor sufficient to ensure replication in cells which do not have YB-1 in the nucleus. It is preferred that the transactivation does not occur in cells which do not have YB-1 in the nucleus independent from the cell cycle or which do not have deregulated YB-1.

Furthermore, these viruses are based on the surprising finding that an adenovirus is capable of replicating in a particularly efficient manner if it comprises at least a nucleic acid which codes for a protein, whereby the protein is selected from the group comprising EIB proteins, E4 proteins and E1A proteins and that at least one protein thereof is under the control of a promoter which is different from the promoter which controls the expression of the respective protein in a wild type adenovirus. Such replication is particularly efficient and usually results in tumor lysis in case the cells have YB-1 in the nucleus, in particular have YB-1 in the nucleus independent of the cell cycle, or in case the cells comprise deregulated YB-1, in particular comprise deregulated YB-1 in the cytoplasm. What has been said above about the
E1B proteins, E4 proteins and E1A proteins applies also here. In wild type adenoviruses the E1B protein is controlled by the E1B promoter, the E4 protein is controlled by the E4 promoter and the E1A protein is controlled by the E1A promoter. By selecting promoters which are different from those which control the expression of the aforementioned proteins in wild type adenoviruses, the expression of the previously mentioned proteins and thus the regulatory interplay of the individual adenoviral nucleic acids and proteins is changed. By selecting the promoters a chronologically different expression pattern can be created which, without wishing to be bound thereto in the following, results in the observed replication in cells, whereby the mechanism may be the one as already previously described with regard to the chronologically different expression of the adenoviral proteins E1B, E4 and E1A. An example of a specific design for the control of said proteins through promoters different from those controlling the expression of the respective proteins in wild type adenovirus, may be taken from the sub-claims and from the example part, whereby in particular the viruses referred to therein as XVirPSJL1 and XVirPSJL2 are representative thereof. Preferably, the E1B protein is the E1B55kD protein, the E4 protein is the E4orf6 protein and the E1A protein is the E1A12S protein.

The promoters which preferably control the E1B protein as well as the E4 protein, are selected from the group comprising tumor-specific promoters, organ-specific promoters, tissue-specific promoters, heterologous promoters and adenoviral promoters under the proviso that when adenoviral promoters are used, they are different from the E1B promoter in case of the expression control of the E1B protein, and are different from the E4 promoter in case of expression control of the E4 protein. The use of the E1A promoter for the expression control of the E1B protein and/or the E4 protein is particularly preferred. The E1A promoter is, for example, described by Boulanger P. A. and Blair, G. E. Biochem. J. 1991, 275, 281-299. Additionally, also the use of each and any other heterologous promoter is possible, i.e. a promoter which is different from the one which controls the expression of the respective protein in a wild type adenovirus. A representative example is the CMV promoter, whereby other promoters will be obvious for the ones skilled in the art.
The promoter which is used for the control of the E1A protein, may also be selected from the group comprising tumor-specific promoters, organ-specific promoters, tissue-specific promoters, heterologous promoters and adenoviral promoters under the proviso that the adenoviral promoter is different from the E1A promoter. It is within the present invention that one or several of the aforementioned proteins, i.e. the E1B protein, the E4 protein or the E1A protein are under the control of the same promoter, whereby it is nevertheless preferred that particularly the E1B protein and the E4 protein are under the control of the same promoter. It is particularly preferred that the expression of the E1A protein is controlled by a YB-1-controlled promoter or a promoter which can be regulated by YB-1. Such promoters are disclosed herein in connection with other aspects of the present invention. The use of the adenoviral E2-late promoter is particularly preferred for the control of the expression of the E1A promoter as it can, first, be regulated by YB-1 and, second, shows only little transcription in the absence of YB-1 which can factually be neglected so that a very good expression control of the nucleic acid which is under the control of the E2-late promoter, is ensured. This considerably increases biological safety, particularly when applied in the field of medicine.

Furthermore, it has been found that adenoviruses will replicate particularly well in cells which have YB-1 in the nucleus, particularly have YB-1 in the nucleus independent of the cell cycle, and/or which have deregulated YB-1, preferably have deregulated YB-1 in the cytoplasm, if YB-1 is provided for replication either directly or indirectly in particular in the cellular nucleus or if the provision of YB-1 is directly or indirectly mediated through an adenoviral protein, whereby such adenoviral protein is different from E1A. This aspect of the present invention is different from the aspect which is also disclosed herein, namely that the use of transactivating E1A-modified adenoviruses, preferably group II adenoviruses, allows for replication of these viruses in YB-1 nucleus-positive tumor cells, particularly YB-1 nucleus-positive cells which are YB-1 positive independent of the cell cycle, and those cells which have deregulated YB-1, particularly comprise YB-1 in the cytoplasm, insofar that the transactivating characteristics of the E1A protein, particularly the E1A13S protein are not used here, i.e. in connection with the group I adenoviruses, but rather in a preferred embodiment the E1A13S protein is functionally inactive and is thus no longer capable of transactivating also E4orf6 and ElB55k, which are involved in the transport and provision of
YB-1, respectively, in the nucleus, either directly or indirectly. Consequently, an effective replication of the adenovirus is not possible in accordance with this aspect of the present invention. Insofar, the provision of YB-1 in the nucleus and the provision of YB-1 for adenoviral replication, respectively, is now no longer under the control of the direct or indirect involvement of the E1A protein but occurs through the expression of the E1B protein, particularly ElB55kD protein, and/or the E4 protein, particularly the E4orf6 protein, which is not controlled by E1A.

This embodiment of the adenovirus may also be provided by one of the above-described measures, for example by bringing forward the chronological expression of the E1B protein and/or the E4 protein compared to the expression of the E1A protein, or by putting one or several of the E1B proteins, E4 proteins and E1A proteins under the control of a promoter which is different from the promoter which controls the expression of the respective protein in wild type adenovirus.

Finally, this design principle of adenoviruses starts from the surprising finding that an effective adenoviral replication may also occur, particularly in cells which have YB-1 in the nucleus, more particularly YB-1 in the nucleus independent of the cell cycle, or in cells which have deregulated YB-1, preferably in the cytoplasm, in case at least one of the E1B proteins, E4 proteins and E1A proteins, particularly the preferred forms thereof, are expressed in an expression cassette under the control of a promoter. In one embodiment of the present invention basically three expression cassettes each comprising a single one of said proteins are provided. In an alternative embodiment an expression cassette may also comprise two or more of the proteins E1B, E4 and E1A and their derivatives and possible substituents, respectively, particularly in case of E1A12S. What has previously been said in relation to the aspect that the adenoviruses comprise nucleic acids related to proteins E1B, E4 and E1A, is also applicable to the design of the various proteins and the respectively used promoters. When using such expression cassettes it is preferred that proteins and nucleic acids coding therefor in the genome of the wild type adenovirus which correspond to the respective proteins of the expression cassettes, are either completely or partially deleted to ensure that the virus is stable and to avoid recombinations, at least to a bigger extent.
In principle, the expression cassettes can be cloned into each region and each site, respectively, of the adenovirus, whereby preferably one or several of the cassettes are inserted either individually or in combination with each other into the E1 region, the E3 region and/or the E4 region of the virus. It is possible that the nucleic acids of the E1, E3 and E4 region are completely deleted, partially deleted or not deleted at all, whereby it is preferred with regard to the adenoviruses according to the invention that the nucleic acid coding for the E1A13S gene is inactivated or deleted so as not to provide any transactivating E1A protein by the virus. The extent of such deletion in one or several of the regions E1, E3 and E4 is determined by the expression cassette used and, optionally, further introduced foreign genes or transgenes or the further expression cassettes comprising them, i.e. genes which are different from the adenoviral genes, at least different in the sense that they are not provided in the regulatory context of the adenoviral nucleic acid as prevailing in wild type adenovirus or are not provided in the sequence of the adenoviral nucleic acids of wild type adenoviruses at such site. It is within the present invention that the nucleic acids which are contained in one or several of the expression cassettes which code for an EIB protein, an E4 protein and/or an E1A protein, are partially or completely deleted in the adenoviral genome. In an embodiment, such as in the adenovirus according to the present invention XvirPSJL 1 or 2, the adenoviral nucleic acid coding for E4orf6 is partially or completely deleted, however, the complete nucleic acid coding therefor is contained in the expression cassette. Preferably, this will also be realised for the ElB55k (also referred to as E1 55Kd) protein and/or the E1A12S protein. The extent of the deletion is to be selected in preferred embodiments such that a maximum package size of about 103 % of the maximum package size of the wild type adenovirus is reached, although this limit is only a preferred limit. The possible deletions to be made in the adenoviral genome are only subject to limitations in preferred embodiments such as to make sure that still infectious and packed particles can be manufactured. The precise extent of the deletions may be determined by the ones skilled in the art on the basis of the disclosure provided herein together with standard tests.

As a starting point for the construction of the adenoviruses described herein, any wild type adenovirus may be used, but also other adenoviruses may be used provided that they are constructed in accordance with the technical teaching of the present invention. It is
particularly preferred to have recourse to adenoviruses of subgroup C and within this group in turn to adenovirus 2 and adenovirus 5.

The terms E1B protein and E1B proteins, E4 protein and E4 proteins as well as E1A protein and E1 proteins are used herein in a synonymous manner, if not indicated to the contrary.

As used herein, the term "deregulated" YB-1 refers to a YB-1 molecule or YB-1 protein as described herein which is present in a form which is quantitatively and/or qualitatively different from YB-1 as normally present in cells, preferably in non-tumor cells. A deregulated YB-1 can be characterised and identified as such by particular viruses being able to replicate in the presence of deregulated YB-1 in a cellular background comprising such deregulated YB-1. The particular viruses in connection therewith are those the E1A protein of which is mutated and exhibits a transactivating function. Examples for these particular viruses are AD delta 24, dl 922-947, E1 Ad/01/07 and CB 016 and/or those described by Howe, J. A et al., Molecular Therapy 2, 485-495, 2000; Fueyo J. et al., Oncogene 19, 2-12, 2000; Heise C. et al., Nature Medicine 6, 1134-1139, 2001; Balague, C et al., J. Virol. 75, 7602-7611, 2001; Bautista, D.S. et al., Virology 1991, 182, 578-596; Jelsma T.N. et al., Virology 1988, 163, 494-502; Wong, H. K. and Ziff E.B., J. of Virology 1994, 68, 4910-4920]. Such a cell and a cell, respectively, having such a background can be used for the replication of group I adenoviruses and/or group II adenoviruses. Additionally, tumors comprising such cells may be lysed by the adenoviruses according to the invention.

Furthermore, this design principle of adenoviruses is based on the surprising finding that the DNA replication of E1A-modified adenoviruses in YB-1 nucleus-positive tumor cells is based on the activation of the E2-late promoter. E1A-modified adenoviruses are to be understood as those which (a) have, in YB-1 nucleus-negative cells, a reduced or no replication at all compared to wild type, (b) have a transactivation activity on at least one viral gene, whereby the gene is particularly selected from the group comprising E1B-55kDa, E4orf6, E4orfG and E3ADP, and/or (c) do not translocate cellular YB-1 into the nucleus by the adenovirus. Optionally, the adenoviruses used in accordance with the present invention have the further characteristic that the binding of the E1A protein encoded by the adenovirus is interfering with the binding of E2F to RB and is capable of dissolving the respective complex consisting
of E2F and Rb. Adenoviruses which have one or several of the aforementioned features a) to c), preferably all of the features a) to c), are replication deficient in cells which do not have YB-1 in the nucleus.

Without wishing to be bound thereto, it is assumed that the E2-early promoter, i.e. the early E2 promoter, is not switched on by means of the human cellular E2F transcription factor in connection with the replication of the viruses used in accordance with the present invention and in connection with the use in accordance with the present invention of the adenoviruses of the present invention. Under such circumstances the start of the replication is independent of the Rb status of the cells, i.e. the tumor cells which are infected by using the viruses disclosed herein and which are preferably lysed subsequently, may contain either functional as well as inactive Rb proteins. In addition, adenoviral replication using the adenoviruses disclosed herein or using the conditions disclosed herein, does not require any functional p53 protein, however is neither negatively affected by its presence. Insofar the technical teaching turns away from the principle underlying the use of oncolytic or tumorlytic adenoviruses of the type of AdA24, dll922-947, E1Ad/01/07, CB016 or those adenoviruses described, for example, in European patent EP 0 931 830, which had been made subject to one and/or several deletion(s) in the E1A protein under the assumption that intact functional Rb proteins would hinder an efficient in vivo replication and thus provide for adenoviral replication in vivo only in Rb-negative and Rb-mutated cells. These adenoviral systems of the prior art are based on E1A in order to control in vivo replication of adenoviruses by means of the early E2 promoter (E2-early promoter) and "free E2F". Nevertheless, these known viruses of the prior art may be used in accordance with the present invention for the replication in cells which contain YB-1 in the nucleus independent of the cell cycle, or in cells which comprise deregulated YB-1.

The viruses and in particular adenoviruses described in said European patent EP 0 931 830 may be used in accordance with the present invention. More specifically, the viruses described in said patent are viruses which are replication deficient and which lack an expressed viral oncoprotein which is capable of binding a functional Rb tumor suppressor gene product. The adenovirus can particularly be any adenovirus which lacks expressed viral E1A oncoprotein which is capable of binding a functional tumor suppressor gene product,
more particularly Rb. The viral E1A oncoprotein can exhibit an inactivating mutation, for example in the CR1 domain at the amino acid positions 30 to 85 in adenovirus Ad5, which is also referred to herein as Ad5, Ad 5, the nucleotide positions 697-790 and/or the CR2 domain at amino acid positions 120 to 130 in Ad 5, the nucleotide position 920 to 967 which are involved in the binding of pl05 Rb protein, pl30 and pl07 protein. However, it is within the present invention that the adenovirus is of type 2 dl 312 or type 5 NT dl 1010.

A further feature of a part of the adenoviruses to be used in accordance with the present invention which are different from the adenoviruses of the present invention, is that they code for a viral oncogene which is also referred to herein as oncogene protein, whereby the oncogene protein is preferably E1A and whereby the oncogene protein is capable of activating at least one viral gene which has an impact on the replication of the virus and/or cell lysis of the cell infected by said virus. Preferably, the impact on the replication is such that the virus replicates better in the presence of the oncogene protein compared to the scenario where the oncogene protein of the respective virus is absent. This process is also referred to herein as transactivating and particularly as E1A transactivating in case the transactivation is mediated by E1A. The term "transactivate" or "transactivation" preferably describes the process that the respective viral oncoprotein has an impact on the expression and/or on the transcription of one or several other genes which are different from the gene coding for the viral oncogene protein itself, i. e. controls its/their expression and/or translation and particularly activates it/them. Such viral genes are preferably ELB55kDa, E4orf6, E4orf3 and E3ADP as well as any combination of the aforementioned genes and gene products, respectively.

A further, although only optional feature of the adenoviruses to be used in accordance with the present invention as well as of the adenoviruses of the present invention is their binding characteristics and the binding characteristics of particular ones of the proteins coded by them, respectively, to tumor suppressor Rb. Basically, it is within the present invention that the adenoviruses used in accordance with the present invention may or may not bind to Rb. The use of any of the two alternative embodiments of the adenoviruses is independent of the Rb status of the cells treated or the cells to be treated.
In order to confer to E1A the ability not to bind to Rb, the following deletions can be made to the E1A oncoprotein: deletion in the CR1 region (amino acid positions 30 - 85 in Ad5) and deletion of the CR2 region (amino acid positions 120 - 139 in Ad5). In doing so, the CR3 region is preserved and can exercise its transactivating function on the other early viral genes.

In order to confer to E1A the ability to bind to Rb, the following deletions to E1A oncoprotein, however, are basically possible: deletion of the CR3 region (amino acid positions 140 - 185); deletion of the N-terminus (amino acid positions 1 - 29); deletion of the amino acid positions 85 - 119; and deletion of the C-terminus (amino acid positions 186 - 289). The regions listed above do not interfere with the binding of E2F to Rb. The transactivating function remains intact, however, is reduced compared to wild type Ad5.

It is also within the present invention, particularly with regard to the adenoviruses of the present invention, that the E1A protein, particularly the E1A12S protein is designed such that, in an embodiment, it is capable of binding to Rb and, in a different embodiment, is not capable of binding to Rb, whereby such E1A12S protein is an E1A protein and particularly an E1A12S protein in the meaning of the present invention which is nevertheless referred to in the prior art sometimes as modified E1A12S. The respective design of the E1A12S protein is within the skills of those of the art, particularly with regard to the aforementioned deletions of the E1A protein which is also referred to herein simply as E1A.

Such adenoviruses which are basically already known in the prior art and which do not show any transactivation, are generally regarded as replication deficient. However, it is the merit of the present inventor that he has recognised that they are nevertheless capable of replicating in a suitable background, in particular a cellular background. Such suitable cellular background is caused or provided by the presence of YB-1 in the nucleus, preferably a cell cycle independent presence of YB-1 in the nucleus, or by deregulated YB-1. The term cells or cellular systems as used herein in connection with each and any other aspect of the present invention, comprises fragments or fractions of cell extracts as well as cells which are present in vitro, in vivo or in situ. Insofar, the term cellular systems or cells also comprises cells which are present in cell culture, tissue culture, organ culture or in any tissue or organ in vivo and in situ, respectively, isolated, in groups or as part of tissues, organs or organisms, but
which may also be present as such in a preferably living organism. The organism is preferably any vertebrate organism and more preferably a mammal. More preferably the organism is a human organism. Other preferred organisms are those disclosed in connection with the various aspects of the present invention.

In connection with the present invention the modified E1A oncoprotein of the various adenoviruses to be used in accordance with the present invention is, in contrast to the viruses of the present invention, capable of transactivating the early viral genes such as E1B55K, E4orf0, E4orf6, E3ADP in YB-1 nucleus-positive cells or cells which comprise deregulated YB-1. There are preferably no other changes made to the viral genome and the respective adenovirus may insofar correspond otherwise to a wild type adenovirus or a derivative thereof.

The viruses disclosed herein which code or comprise a transactivating oncogene protein in the meaning of the present invention, comprise, for example, the adenoviruses AdA24, dl922-947, ElAd/01/07, CB106 and/or the adenoviruses described in European patent EP 0 931 830 which are each capable of transactivating the early genes such as EIB, E2, E3 and/or E4 and which are comparable to the adenoviruses of wild type, particularly wild type Ad5. In these cases, a distinct region of the E1A protein is responsible for the transactivation. Within the various adenoviral serotypes there are three highly conserved regions within the E1A protein. The region CR1 from amino acid positions 41 - 80, CR2 from amino acid positions 120 - 139 and CR3 from amino acid positions 140 - 188. The transactivating function is mainly based on the presence of the CR3 region within the E1A protein. The amino acid sequence of CR3 is present in an unchanged manner in the above mentioned adenoviruses. This results in a transactivation of the early genes EIB, E2, E3 and E4 independent of whether YB-1 is present in the nucleus or in the cytoplasm.

In contrast thereto, the CR3 region has been deleted in the recombinant adenovirus dl520. Thus, dl520 expresses a so-called E1A12S protein which does not comprise the amino acid sequence of the CR3 region. Consequently, dl520 may exercise only a very weak transactivating function, particularly on the E2 region, and thus does not replicate in YB-1 nucleus-negative cells. In YB-1 nucleus-positive cells YB-1 is responsible for the
transactivation of the E2 region and thus allows for an efficient replication of dl520. The use of systems like dl520 or systems originating therefrom for the purposes disclosed herein, is based thereon. A further important difference between the two previously described groups of adenoviruses such as, for example, delta 24 (also referred to herein as AdA24) and, for example, dl520, resides in the fact that the early genes E1B, E3 and E4 are more comprehensively transactivated in cells being YB-1 nucleus-positive cells independent of the cell cycle or in cells containing deregulated YB-1, compared to YB-1 nucleus-negative cells or cells which do not comprise deregulated YB-1. In contrast thereto, there are no or only minor differences in delta 24. The transactivation of dl520, more specifically of the E1A12S protein is, however, significantly reduced compared to wildtype adenovirus. This transactivation, however, is sufficient so as to provide for an efficient replication in YB-1 nucleus-positive cells. The design of the E1A protein as described herein and in particular as described in this connection, and of the nucleic acid coding therefor, such that the E1A protein has, compared to the wild type oncogene protein E1A, one or several deletions and/or mutations, including and particularly preferably those designs of the E1A protein as described in connection with dl520 or AdA24, dl922 to 947, E1Ad/01/07, CB106 and/or the adenoviruses described in European patent EP 0 931 830, are embodiments of viruses, in particular of adenoviruses, the replication of which is controlled, preferably predominantly controlled by the activation of the E2-late promoter. Preferably, the deletion is such that it is selected from the group comprising deletions of the CR3 region and deletions of the N-terminus and deletions of the C-terminus. Further embodiments of the E1A protein which allow this kind of replication of adenoviruses, can be generated by the ones skilled in the art based on the disclosure provided herein. The embodiment of the E1A protein as described previously is an embodiment which may also be used in connection with the adenoviruses of the present invention which are also referred to herein as adenoviruses of the present invention or group I adenoviruses.

The adenoviruses of the present invention, particularly the group I adenoviruses, which are also referred to herein as derivatives and which may be used in accordance with the present invention, typically comprise an E1 deletion, an E1/E3 deletion and/or an E4 deletion, i.e. the corresponding adenoviruses are not capable of generating functionally active E1 and/or E3 and/or E4 expression products and corresponding products, respectively. Or in other words
these adenoviruses are only capable of generating functionally inactive E1, E3 and/or E4 expression products, whereby a functionally inactive E1, E3 and/or E4 expression product is an expression product which is either not present as an expression product at all, either at the transcription level and/or at the translation level, or is present in a form which at least does not have one of the functions attributed to it in a wild type adenovirus. This/these function(s) inherent to the expression product in wild type adenovirus is/are known to the ones skilled in the art and, for example, described in Russell, W. C., Journal of Virology, 81, 2573-2604, 2000. Russell (supra) also describes design principles of adenoviruses and adenoviral vectors which are incorporated herein by reference. It is also within the present invention that the modified E1A oncoprotein, i.e. the no longer transactivating E1A protein and other proteins such as E1A12S, E1B-55K, E4orf6 and/or E3ADP (adenoviral death protein (ADP)) (Tollefson, A. et al., J. Virology, 70, 2296-2306, 1996) are expressed in such vector either alone or in any combination. The individual mentioned genes as well as the transgenes disclosed herein, may be, independently from each other, cloned into the E1 and/or E3 and/or E4 region and expressed using a suitable promoter or under the control of a suitable promoter. Basically, each of the E1, E3 and E4 region is suitable as cloning site within the adenoviral nucleic acid, whereby the region which is not used for the cloning can either be present, or partially and/or completely deleted. In case these regions are present, in particular are completely present, it is within the present invention that these are either intact and preferably provide a translation product and/or a transcription product, and/or are not intact and preferably do not provide a translation product and/or transcription product. In embodiments suitable promoters are those as disclosed herein in connection with the controlling and expression, respectively, of E1A, in particularly of the modified E1A.

Finally, in an embodiment, the group II adenoviruses used in accordance with the present invention are E1B deficient, particularly E1B 19 kDa deficient. The term deficient as generally used herein refers to a condition, wherein the E1B does not exhibit all of the characteristics of the wild type E1B and lacks at least one of these characteristics.

The adenovirus BCL2-homologue E1B19k avoids the E1A induced apoptosis by interaction with the pro-apoptotic proteins Bak and Bax. Because of this a maximum replication and/or particle formation is possible in infected cells (Ramya Sundararajan and Eileen White,
Journal of Virology 2001, 75, 7506-7516). The absence of E1B 19k results in a better release of virus as, if present, it assumingly minimizes the function of the adenoviral death protein. The virus induced cytopathic effect is increased by such deletion (Ta-Chiang Liu et al., Molecular Therapy, 2004) and thus results in a more pronounced lysis of infected tumour cells. Additionally, the absence of E1B 19k causes that TNF-alpha does not have any effect on the replication of such adenoviruses in tumour cells whereas in normal cells the treatment results in a less pronounced replication and release of infectious virus. Insofar both selectivity and specificity are increased (Ta-Chiang Liu et al., Molecular Therapy, 2004, 9, 786-803).

At least some embodiments of the group II adenoviruses as used in accordance with the invention disclosed herein, are as such known in the art. The adenoviruses used in accordance with the invention are preferably recombinant adenoviruses, particularly also if, compared to the wild type, a change has been made in the sense of the technical teaching provided herein. It is within the skills of those of the art to delete and mutate, respectively, the adenoviral nucleic acid sequences which are irrelevant for the invention. Such deletions may be related to, e. g. a part of the E3 and E4 coding nucleic acids as also described herein. A deletion of E4 is particularly preferred provided that such deletion does not extend to the protein E4orf6, in other words the adenovirus to be used in accordance with the invention codes for E4orf6. In preferred embodiments, these adenoviral nucleic acids may still be packed into viral capsids and thus form infectious particles. This is also true for the use of the nucleic acids in accordance with the invention. Generally it is also to be acknowledged that the adenoviral systems may be deficient with regard to single or several expression products. In connection therewith it is to be taken into consideration that this, in connection with both the group I adenoviruses and the group II adenoviruses, may be caused by the mutation or deletion of the nucleic acid coding the expression product, whereby such mutation and deletion, respectively, is either a complete one or performed to the extent that no expression product is formed anymore or by the regulatory elements and elements controlling the expression such as promoters and transcription factors being missing or being active in a way different from wild type, either at the level of the nucleic acid (lack of a promoter; cis acting elements) or at the level of the translation and transcription system (transacting elements), respectively. Particularly the latter aspect may depend on the respective cellular background.
Apart from using adenoviruses which are as such already known, in accordance with the present invention also novel adenoviruses such as group II adenoviruses may be used for the purposes already disclosed for the other adenoviruses described herein. The new adenoviruses of the invention result from the technical teaching provided herein. Particularly preferred representatives are, for example, the viruses Xvir03 and Xvir03/01.

In case of vector Xvir03 a CMV promoter was cloned into the E1 region which controls the nucleic acids for E1B 55k and E4orf6 which are separated by an IRES sequence. In connection therewith, the E3 region and the E4 region can be deleted and/or be present and intact. Due to the cloning of these two genes into the virus and due to the gene products generated therefrom, respectively, a high replication efficiency results which actually corresponds to the one of wild type viruses, whereby the selective replication in cells, preferably tumor cells, is maintained insofar as a replication occurs particularly in YB-1 nucleus-positive cells and more particularly in those cells which comprise deregulated YB-1 in the sense of the present disclosure. Cells in which deregulated YB-1 is present are, in an embodiment, cells which show an increased expression of YB-1, preferably compartment independent expression of YB-1, compared to normal or non-tumour cells. However, the introduction of E1B 55k and E4orf6 by cloning can also be made into the E4 region, whereby the E3 region can be either intact or can be deleted.

A further development of virus Xvir03 is virus Xvir03/01 into which in a preferred embodiment therapeutic genes or transgenes have been cloned under the control of a specific promoter, in particular a tumor-specific or tissue-specific promoter. In connection therewith the E3 and E4 region can be deleted and/or be resent and intact. In connection with such virus also the E4 region is functionally inactive, is preferably deleted. The transgenes described herein may also be cloned into the E4 region, whereby this can be done either alternatively or in addition to the cloning of the transgenes into the E3 region.

The transgenes described herein and particularly described in the following, may also be expressed in connection with or by the adenoviruses of the present invention, i.e. group I adenoviruses and their nucleic acids, respectively, or the replication systems of the invention and are thus comprised in connection with an expression cassette comprising a promoter and a
nucleic acid sequence, whereby such nucleic acid sequence codes for one or several of said transgenes. The El, E3 and/or E4 regions are particularly suitable cloning sites in the adenoviral genome, however, the cloning sites are not limited thereto. Transgenes, as used herein, may be therapeutic genes or viral genes, preferably adenoviral genes, which, preferably, are not contained in the genome of wild type adenovirus or are not present at the site in the genome where they are present now in the particular virus.

The nucleic acid coding for YB-1 which may be part of the adenoviruses in an embodiment of the adenoviruses to be used in accordance with the invention, particularly group II adenoviruses, but also of the adenoviruses according to the invention, i.e. group I adenoviruses, may comprise a nucleic acid sequence which mediates the transport of YB-1 into the nucleus. The nucleic acids, adenoviruses and adenoviral systems according to the invention as well as the adenoviruses known in the prior art such as, for example, Onyx-15, AdA24, dl922-947, E1Ad/01/07, CB016, dl 520 and the adenoviruses described in patent EP 0 931 830 may be used, as adenoviruses and adenoviral systems, respectively, and the corresponding nucleic acids, in combination with these nucleic acids in accordance with the invention. Suitable nucleic acid sequences mediating nuclear transport are known to the ones skilled in the art and, for example, described in Whittaker, G.R. et al., Virology, 246, 1-23, 1998; Friedberg, E.C., TIBS 17, 347, 1992; Jans, D.A. et al., Bioassays 2000 Jun; 22(6): 532-44; Yoneda, Y., J. Biochem. (Tokyo) 1997 May; 121(5): 811-7; Boulikas, T., Crit. Rev. Eukaryot. Gene Expr. 1993; 3(3): 193-227; Lyons RH, Mol. Cell Biol., 7 2451-2456, 1987).

The nucleic acid sequences mediating nuclear transport may realise different principles. One such principle is that YB-1 forms a fusion protein with a signal peptide or is provided with such signal peptide and is transferred into the cellular nucleus because of the signal peptide, whereupon the replication of the adenoviruses in accordance with the invention occurs.

A further principle which may be used in the design of the adenoviruses to be used in accordance with the invention, particularly group II adenoviruses, but also with the adenoviruses in accordance with the present invention, i.e. the group I adenoviruses, is providing YB-1 with a transport sequence which results in the transfer or translocation of YB-1 into the cellular nucleus, preferably starting from a synthesis in the cytoplasm, and prompts viral replication there. An example for a particularly effective nucleic acid sequence
mediating transport into the nucleus, is the TAT sequence of HIV which is, for example, described together with other suitable nucleic acid sequences of that kind in Efthymiadis, A., Briggs, LJ, Jans, DA., JBC 273, 1623-1628, 1998. It is within the present invention that the adenoviruses to be used in accordance with the invention, particularly group II adenoviruses, but also the adenoviruses according to the present invention, i.e. group I adenoviruses, comprise the nucleic acid sequences which code for the peptides which mediate nuclear transport.

It is within the present invention that YB-1 is present in its full length, particularly in a form which corresponds to wild type YB-1. Furthermore, it is within the invention that YB-1 is used or present as a derivative, for example in a shortened or truncated form. A YB-1 derivative as may be used or may be present in connection with the present invention, is a YB-1 which is preferably capable of binding to the E2 late promoter and thus activates gene expression of the adenoviral E2 region. Such derivatives particularly comprise the YB-1 derivatives disclosed herein. Further derivatives can be generated by deletion of single or several amino acids at the N-terminus, the C-terminus or within the amino acid sequence. It is within the present invention that also YB-1 fragments are used as YB-1 proteins in the sense of the present invention. In the paper of Jurchott K et al. [JBC 2003, 278, 27988-27996] various YB-1 fragments are disclosed which are characterised by deletions at the C- and the N-terminus. The distribution of the various YB-1 fragments has shown that both the cold shock domain (CSD) as well as the C-terminus is relevant for the cell cycle regulated transport of YB-1 into the cellular nucleus. It is thus within the present invention that a shortened YB-1 (herein also referred to as YB-1 protein) in connection with the inventive expression of ElB55k and E4orf6 migrates better into the nucleus and thus induces a stronger CPE without necessarily binding better to the E2-late promoter compared to native YB-1, whereby it cannot be excluded that also a shortened YB-1 migrates better into the nucleus and is causing both effects, i.e. induces CPE and binds to the E2-late promoter. Finally, such shortened YB-1 fragments may also migrate better into the nucleus and bind more efficiently to the E2-late promoter without inducing a better CPE. It is also within the present invention that shortened YB-1 proteins and fragments, respectively, comprise further sequences as disclosed herein in connection with the full length YB-1, in particular cell localisation signal sequences (NLS) and the like.
In connection with the present invention it is possible that the adenoviruses used in accordance with the invention, particularly group II adenoviruses, but also group I adenoviruses and the nucleic acids coding therefor, is any respective adenoviral nucleic acid which as such or in combination with further nucleic acid sequences results in a replication event. It is possible, as explained herein, that the sequences and/or gene products necessary for replication are provided by helper viruses. To the extent it is referred to coding nucleic acid sequences and said nucleic sequences are nucleic sequences which are known, it is within the present invention that not only the identical sequence is used but also sequences derived therefrom. Herein, derived sequences shall mean in particular any sequences which still result in a gene product, either a nucleic acid or a polypeptide which has a function which corresponds to a or the function of the non-derived sequence. This can be tested by routine tests known to the one skilled in the art. An example for such derived nucleic acid sequences are those nucleic acid sequences which code for the same gene product, in particular for the same amino acid sequence, which, however, have a different base sequence due to the degeneracy of the genetic code.

With regard to the adenoviruses according to the invention of group II and/or the corresponding adenoviral replication system according to the invention and their use in accordance with the invention, respectively, in an embodiment the adenoviral nucleic acid is deficient for the expression of the oncogene protein, in particular is E1A protein deficient, i.e. does either not code for the 12S E1A protein (herein also referred to as E1A12S protein) or for the 13S E1A protein (herein also referred to as E1A13S protein) or does not code for both the 12S E1A protein and the 13S E1A protein, or is modified, as defined herein, if not indicated to the contrary, and that the adenoviral replication system further comprises a nucleic acid of a helper virus, whereby the nucleic acid of the helper virus comprises a nucleic acid sequence which codes for the oncogene protein, particularly the E1A protein, which has the following characteristics and confers the following characteristics to the adenovirus, respectively: It is preferably non-replicating in YB-1 nucleus-negative cells but is replicating in cells which are independent of the cell cycle in YB-1 nucleus-positive or in cells exhibiting deregulated YB-1, is transactivating at least one viral gene, in particular ElB55kDa, E4orf6, E4orf3 and/or E3ADP, in YB-1 nucleus-positive cells, and/or does not transfer cellular YB-1
into the nucleus. It is within the present invention that the transgenes described herein are either individually or collectively coded and/or expressed by the helper virus. This applies to helper viruses for both group I adenoviruses and group II adenoviruses.

Group I adenoviruses and/or group II adenoviruses, but also virus group #1 and #3, are characterised by the various nucleic acids and gene products, respectively, disclosed herein and may otherwise comprise all those elements known to the ones skilled in the art and which are inherent to the wild type adenoviruses (Shenk, T.: Adenoviridae: The virus and their replication. Fields Virology, vol. 3, editors Fields, B.N., Knipe, D.M., Howley, P.M. et al., Lippincott-Raven Publishers, Philadelphia, 1996, chapter 67).

As already mentioned, group I and/or group II adenoviruses are capable of replicating in such cells and cellular systems, which have YB-1 in the nucleus. For the question whether also these adenoviruses used in accordance with the invention are capable of replicating and are thus capable of tumor lysis, the status of the cells with regard to the presence or absence of Rb, i.e. the retinoblastome tumor suppressor product, is irrelevant. Furthermore, for the use of said adenoviruses in accordance with the present invention, it is not necessary to take into account the p53 status of the infected cells, of the cells to be infected or of the cells to be treated as, when using the adenoviral systems disclosed herein in connection with YB-1 nucleus-positive cells, i.e. cells having YB-1 in the nucleus independent of the cell status, the p53 status as well as the Rb status does not have any impact on the replication of the adenovirus for the practising the technical teaching disclosed herein.

The transactivating oncogene and oncogene protein, respectively, in particular E1A, preferably of the group II adenoviruses, can be either under the control of the proprietary natural adenoviral promoters and/or be controlled through a tumor-specific or tissue-specific promoter. Suitable non-adenoviral promoters can be selected from the group comprising cytomegalovirus promoter, RSV (rous sarcoma virus) promoter, adenovirus-based promoter Va I and the non-viral YB-1 promoter (Makino Y. et al., Nucleic Acids Res. 1996, 15, 1873-1878). Further promoters which may be used in connection with any aspect of the invention disclosed herein, are the telomerase promoter, the alpha-fetoprotein (AFP) promoter, the caecinoembryonic antigen promoter (CEA) (Cao, G., Kuriyama, S., Gao, J., Mitoro, A., Cui,

It is within the present invention that the various promoters described above are also used in connection with the various embodiments of the adenoviruses in accordance with the invention, preferably the group I adenoviruses, particularly in case a promoter is to be used which is different from the one which controls the expression of the respective protein or expression product in wild type adenoviruses. The aforementioned promoters are thus suitable heterologous promoters in the meaning of the present invention. In preferred embodiments of the adenoviruses in accordance with the invention, particularly the group I adenoviruses, it is contemplated that when applying the adenoviruses for cells of group A and B as defined above, this occurs such that the expression of the E1B protein and/or the E4 protein starts from such heterologous promoters, whereby preferably, but not exclusively, the expression of the E1A protein is controlled by YB-1. The expression of the E1A protein is in this and other embodiments under the control of a YB-1 controllable promoter such as for example the adenoviral E2-late promoter. This is also true in that case where the E1B protein and/or the E4 protein is/are expressed in an expression cassette.

In preferred embodiments of the adenoviruses in accordance with the invention, particularly the group I adenoviruses, it is contemplated that when applying the adenoviruses in
connection with cells of group C the promoter is each and independently a tumor-specific, organ-specific or tissue-specific promoter. In connection therewith it is sufficient when at least one of the promoters which control the expression of the E1B protein, the E4 protein and/or the E1A protein, is such a specific promoter. By this tumor, organ and tissue specificity, it is ensured that replication of the adenoviruses in accordance with the invention happens only in cells of the respective tumor, organ or tissue and that, apart from that, no further tissue is damaged by the replication of the adenoviruses such as, for example, is lysed. Preferably, still a second and more preferably all three proteins are controlled by such tumor-specific, organ-specific or tissue-specific promoters. Using such adenoviruses it is possible to lyse also those cells which do not form a tumor or which cannot develop into such tumor, but which are for other reasons such as medicinal reasons to be destroyed or to be removed from the organism, preferably a mammalian and more preferably a human organism, for example because they produce an undesired factor or produce such factor at a too high level.

Virus group #3

This group of viruses is based on the surprising finding that the viruses according to the invention, i.e. viruses which lack a functional E1-region as present wild type adenoviruses, and which at the same time comprise a transporter and in particular code for such transporter which may transport or translocate YB-1 into the nucleus, are capable of replicating in cells which either contain YB-1 in the nucleus in a cell cycle independent manner, or in cells which have a deregulated YB-1.

Furthermore, it has been found that these viruses may also replicate independently of E1A13S, in particularly if the replication is mediated through YB-1. The replication occurs under such conditions in particular in the afore described cells. As used herein, cell which contain YB-1 in the nucleus, preferably contain YB-1 in the nucleus independent of the cell cycle, are also those cells which contain YB-1 in the nucleus due to the use of the viruses in accordance with the present invention and in particular due to the infection of the cells with them.
Finally, it has also been recognized that protein IX is an important factor in particular for the efficacy of the viruses in accordance with the present invention, particularly when used as oncolytic viruses, and that the constructs disclosed herein provide for an expression of this factor which results in a high-level particle formation also in YB-1-mediated E1A13S-independent viral replication.

The viruses in accordance with the present invention comprise a transporter for the transport of YB-1 to the cell nucleus. In a preferred embodiment the transporter is a protein, preferably a viral protein. The YB-1 which is transported into the nucleus of a cell by the transporter, is preferably a deregulated YB-1, in particular as defined herein. However, it is also within the present invention that YB-1 is one that is encoded, alternatively or additionally, to the deregulated YB-1 by the virus in accordance with the present invention and is expressed in the cell which is infected by the virus.

The cells, in which the transporter of the viruses in accordance with the present invention transports YB-1 into the nucleus, are preferably those which contain the regulated YB-1.

It is within the skills of those of the art to assess whether and if so a virus comprises such a transporter or codes therefor. In connection therewith, in one embodiment, a cell which comprises YB-1 in the nucleus in a cell cycle independent manner, such as for example the cervix carcinoma cell line HeLa or the osteosarcoma cell line U20S can be used and subsequently be determined, whether due to the infection and the subsequent replication of the virus the corresponding infected cell contains YB-1 in the nucleus. In an alternative embodiment a cell is used as a cell in connection therewith which contains deregulated YB-1. YB-1 can be detected under such experimental conditions in the nucleus using the means described herein, in particular an antibody directed against YB-1, as can be made by the one skilled in the art. If, under the influence of the virus, YB-1 is detected in the cell nucleus, the tested virus comprises the transporter.

It is within the present invention that the E1A-region is "minus" with regard to one or both protein groups coded by the E1-region in the meaning of the afore-mentioned embodiments. Said two protein groups are the group of E1A proteins, in particular the E1A13S protein, also
referred to herein as E1A13S, and the E1A12S protein, also referred to herein as E1A12S, and the group of EIB proteins, in particular the ElB55k protein, also referred to herein as ElB55k, the EIB 19k protein, also referred to herein as EIB 19k, and the protein IX.

It is within an embodiment of the present invention that the virus is ElA13S-minus if E1A13S is under the control of a promoter which is different from the E1A promoter, preferably the adenoviral ElA-promoter and more preferably the adenoviral E1A-promoter of the wild type; the virus is ElA12S-minus if E1A12S is under the control of a promoter which is different from the E1A promoter, preferably the adenoviral E1A-promoter and more preferably the adenoviral E1A promoter of the wild type. The virus is ElB55k-minus if ElB55k is under the control of a promoter which is different from the EIB promoter, preferably the adenoviral E1A promoter and more preferably the adenoviral EIB promoter of the wild type; the virus is ElB19k-minus if EIB 19k is under the control of a promoter which is different from the EIB promoter, preferably the adenoviral EIB promoter and more preferably the adenoviral EIB promoter of the wild type; and it is protein IX-minus if protein IX is under the control of a promoter which is different from the EIB IX-promoter, preferably the adenoviral EIB IX-promoter and more preferably the adenoviral EIB IX-promoter of the wild type or if it is under the control of the EIB IX-promoter, however said promoter is inactive due to the lack of in particular viral factors which direct the activity of the EIB IX-promoter; the latter is thus an example that the regulatory context is changed, more specifically that the regulatory context is indirectly changed or changed at a higher integration or regulatory level. In general, the term changed regulatory context thus comprises also changes which are either indirectly or at a higher integration or regulatory level active, however, in any case are different from the particularities of the wild type, in particular the wild type adenovirus.

In an embodiment of the present invention the virus is ElA13S-minus. In a further embodiment the virus is also ElA12-minus. In connection therewith it is particularly preferred when the viral E1A12S is under the control of a promoter the activity of which is controlled by YB-1, in particularly is activated by YB-1. These promoters are referred to herein as YB-1-dependent promoters. A particularly preferred YB-1-dependent promoter is the adenoviral E2-late promoter. By this construction it is ensured that E1A12S is activated in
the course of viral replication only when YB-1 is present in the nucleus. This is achieved in case of cells with deregulated YB-1 through the transporter of the viruses in accordance with the present invention, which translocates the deregulated YB-1 into the nucleus of the infected cell. Due to the chronologically reversed expression of the viral transporter and of E1A12S compared to the expression in wild type, E1A12S is specifically only expressed in such cells which contain YB-1 in a deregulated form and thus limit replication of the virus to these cells and, consequently, limit the lysis to particularly these cells which represents a significant advantage of this viral design in terms of safety.

Under these circumstances the particle number in connection with YB-1-dependent replication was to be increased. The present inventor has recognized that protein IX also plays an important role in YB-1 dependent replication and that its expression is not effected by the afore-described chronological change in the expression of the transporter, which is preferably provided by the proteins of the E1B region, and of E1A12S when realizing the designs disclosed herein. The adenoviral designs described in the prior art for YB-1-dependent replication exhibited despite outstanding oncolytic activities a particle formation which was low for some applications which, for example, required a further application of the oncolytic virus. Such further application of viruses is in principle possible, however is not desired in the majority of cases. Particle formation could significantly be improved by the constructs described herein.

The adenoviral protein IX cements the capsid structure and is important for the packaging of viral DNA into virions (Boulanger et al., Journal of Virology, 44, 783-800, 1979; Jones and Shenk, Cell, 17, 683-689, 1979). The gene is located in the viral genome between positions 3581 and 4071 (Colby and Shenk T, Journal of Virology, 1981, 39, 977-980), whereby the gene for protein IX is expressed only from replicating DNA-molecules (Matsui T et al., Molecular and Cellular Biology, 1986, 6, 4149-4154).

Virus Xvir03-3'UTR which is described in the prior art and which performs a YB-1-dependent replication, comprises both the promoter as well as the sequence for protein IX as has been shown in analysis performed in the meantime in connection with the present invention, as the 3'UTR sequence contains the same. However, the protein is only weakly
expressed in tumour cells and results in a comparatively low particle formation compared to wild type virus. The virus Xvir03-3'UTR expresses the viral proteins ElB55k and E4orf6 as mediated by the heterologous CMV promoter (company Clonetech: Plasmid pShuttle) introduced into Xvir03-3'UTR. Rather than the CMV promoter, also all those promoters described herein as disclosed in connection with the expression of E1A may be used. The open reading frames of both genes are linked to each other by means of a so-called IRES sequence (engl. internal ribosomal entry site) (Pelletier, J. and Sonenberg, N. Nature, 1988, 334, 320-325). This element (company Novagen: pCITE) allows the expression of two proteins from one mRNA. A further option for the expression of two proteins from one RNA is the use of short peptides (2A), which are derived from foot and mouth disease virus (Pablo de Felipe, Genetic Vaccines and Therapy, 2004, 2, 13). This element can in principle be used as an alternative to the regulatory IRES sequence in the various embodiments described herein.

From the regulatory background of the expression of protein IX in YB-1-dependency replicating viruses and in particular adenoviruses, which has been unknown prior to the present application, the present inventor recognized that the expression of protein IX in connection with YB-1-dependent replication and in case of viruses which replicate in a YB-1-dependent manner, can basically be provided by the following different strategies:

1. By means of an independent promoter which is directed by protein E1A12S or by protein E1B19L

The independent promoter is preferably a promoter which is different from the E1B IX promoter. Preferably the independent promoter is selected from the group comprising tissue-specific, tumour-specific, YB-1-dependent and viral promoters.

2. Controlling the expression of protein IX by E1A12S. The induction of the S phase in an infected cell occurs by the expression of the E1A12S protein which results in protein IX being activated by its natural promoter.
It is within the present invention that in principle such promoters are used for the expression of the transporter which are different from the promoter which controls the expression of the transporter in the wild type virus. In preferred embodiment this means that E1B55k is controlled by a promoter different from E1B, and E4orf6 by a promoter different from the E4 promoter. In a further embodiment the promoter is a promoter which is E1A independent, i.e. its activity is not influenced by E1A. Preferred promoters are thus preferably tissue-specific promoters, tumour-specific promoters and viral promoters, in particular those described herein.

YB-1 dependent promoters which can be used within the present invention comprise, but are not limited to, the adenoviral E2-late promoter, the MDR-promoter [Stein et al, J. Biol. Chem, 2001, 276, 28562-28569] as well as the DNA polymerase alpha-promoter [En-Nia et al, J. Biol. Chem., 2004, Epub ahead of print].

Suitable non-adenoviral promoters which are useful within the present invention, can be selected from the group coprising cytomegalovirus promoter, RSV-(Rous sarcoma Virus)-Promotor, adenovirus-based promoter Va I and the non-viral YB-1-promoter (Makino Y. et al., Nucleic Acids Res. 1996, 15, 1873-1878). Further promoters which may be used in connection with any aspect of the invention disclosed herein, are the telomerase promoter, the alpha-fetoprotein (AFP)-promoter, the caecinoembryonic antigen promoter (CEA) (Cao, G., Kuriyama, S., Gao, J., Mitotor, A., Cui, L., Nakatani, T., Zhang, X., Kikukawa, M., Pan, X., Fukui, H., Qi, Z. Int. J. Cancer, 78, 242-247, 1998), the L-plastin-promoter (Chung, I., Schwartz, PE., Crystal, RC, Pizzorno, G, Leavitt, J., Deisseroth, AB. Cancer Gene Therapy, 6, 99-106, 1999), arginine-vasopressin-promoter (Coulson, JM, Staley, J., Woll, PJ. British J. Cancer, 80, 1935-1944, 1999), E2f-promoter (Tsukada et al. Cancer Res., 62, 3428 - 3477), uroplakin II promoter (Zhang et al., Cancer Res., 62, 3743-3750, 2002) and the PSA promoter (Hallenbeck PL, Chang, YN, Hay, C, Golightly, D., Stewart, D., Lin, J., Phipps, S., Chiang, YL. Human Gene Therapy, 10, 1721-1733, 1999). Furthermore the YB-1 dependent E2-late promoter of adenoviruses as disclosed in German patent application DE 101 50 984.7 is a promoter which may be used within the present invention.
The viruses in accordance with the present invention allow for a significantly increased particle formation compared to YB-1 dependent viruses of the prior art. Preferably, the particle formation is increased by a factor of 2 to 50, more preferably by a factor of 10 to 50.

Finally, in a preferred embodiment, the adenovirus used in accordance with the invention is deficient with regard to E1B, in particular E1B19k deficient. As generally used herein, the term deficient refers to a condition in which E1B does not exhibit the entirety of characteristics inherent to the wild type and at least one of these characteristics is lacking. The adenovirus BCL2-homologoue E1B19k prevents the E1A induced apoptosis by interaction with the pro-apoptotic proteins Bak and Bax. Thus the maximum replication and/or particle formation is possible in infected cells (Ramya Sundararajan und Eileen White, Journal of Virology 2001, 75, 7506-7516). The absence of E1B19k results in a better release of the viruses as, if present, minimizes the function of the adenoviral death protein. The virus induced cytopathic effect is increased by such deletion (Ta-Chiang Liu et al., Molecular Therapy, 2004) and results in a stronger lysis of the infected tumour cells. Additionally, the absence of E1B19k results in TNF-alpha not having any effect on the replication of such recombinant adenoviruses in tumour cells, whereas the treatment results in a reduced replication and release of infectious viruses in normal cells. Thus the selectivity and specificity are increased (Ta-Chiang Liu et al., Molecular Therapy 2004, 9, 786-803).

The following aspects, features and embodiments are applicable to all of the viruses used in accordance with the present invention and to be used in accordance with the present invention, in particular adenoviruses.

As used herein, the term transgene comprises in an embodiment all those genes which are either not contained in the virus, in particular the adenovirus of wild type and more preferably adenovirus Ad5 wild type, or contained in a different regulatory context, as defined herein. It is within an embodiment of the present invention that one or several of the transgenes as described herein are coded and/or expressed by one or several helper genes.
The findings described herein and the methods, uses or nucleic acids, proteins, replication systems and the like, respectively, are not necessarily limited to adenoviruses. In principle such systems also exist in other viruses which are herewith also encompassed.

When using the viruses in accordance with the present invention or when using in accordance with the present invention the viruses described herein a replication comparable to the one of the wild type can preferably already be realized with an infection number of 1 to 10 pfu/cell compared to 10 to 100 pfu/cell according to the prior art.

The viruses used in accordance with the present invention preferably provide for a significantly increased particle formation compared to the YB-1-dependent viruses of the prior art. Preferably, the particle formation is increased by a factor of 2 to 50, more preferably by a factor of 10 to 50.

It is within the skills of those of the art to delete and mutate, respectively, the adenoviral nucleic acid sequences which are irrelevant for the invention. Such deletions can, for example, be related to a part of the E3 and E4 coding nucleic acid as also described herein. In case of a deletion of E4 it is particularly preferred if it does not extent to the protein E4orf6, which means that the adenovirus to be used in accordance with the present invention codes for E4orf6. In preferred embodiments these adenoviral nucleic acids may still be packed into the viral capsid and thus form infectious particles. This is also true for the use of the nucleic acids in accordance with the present invention. In general it is to be noted that the adenoviral systems may be deficient with regard to single or several expression products. In connection therewith it is to be taken into consideration that this may, on the one hand, be based on the fact that the nucleic acid coding the expression product is completely mutated or deleted or to the extent mutated or deleted that essentially no expression product is formed any more or that the regulatory and the expression controlling elements such as promoters or transcription factors are lacking or are active in a manner different from wild type, either at the level of the nucleic acid (lack of promoter; cis-acting elements) or at the level of the translation or transcription system (trans-acting elements). In particular the last aspect may depend on the respective cellular background.
A further principle which may be realised in the design of the adenoviruses used in accordance with the invention, is that YB-1 can be provided with a transporter sequence which, preferably starting from synthesis in the cytoplasm, introduces YB-1 into the cell nucleus or which translocates YB-1 into the cell nucleus, and promotes viral replication there. An example for a particularly effective nucleic acid sequence mediating nucleus transport is the TAT sequence of HIV which is, among other suitable nucleic acid sequences of that type described in Efthymiadis, A., Briggs, LJ, Jans, DA., JBC 273, 1623-1628, 1998. It is within the present invention that the adenoviruses which are used in accordance with the present invention, comprise nucleic acid sequences which code for peptides coding for nuclear transportation.

It is within the present invention that YB-1 is present in its full length, particularly in a form which corresponds to the wild type of YB-1. It is within the present invention that YB-1 is used or present as a derivative, such as, e. g. in shortened or truncated form. A YB-1 derivative as used or present within the present invention, is a YB-1 which is capable of binding to the E2-late promoter and thus activates gene expression of the adenoviral E2 region. Such derivatives particularly comprise the YB-1 derivatives disclosed herein. Further derivatives may be generated by deletion of single or several amino acids in the N-terminus, at the C-terminus or within the amino acid sequence. It is within the present invention that YB-1 fragments also used as YB-1 proteins in the meaning of the present invention. Various YB-1 fragments are disclosed in the paper of Jurchott K et al. [JBC 2003, 278, 27988-27996] which are characterized by deletions in the C-terminus and the N terminus. The distribution of the various YB-1 fragments indicated that both the cold-shock domain (CSD) as well as the C-terminus are important for the cell cycle-regulated transport of YB-1 into the nucleus. It is thus within the present invention that a truncated YB-1 (which is also referred to herein as YB-1 protein) is migrating in a better way into the nucleus in combination with the expression of ElB55k and E4orf6 in accordance with the present invention and thus induces a stronger CPE without necessarily binding better to the E2-late promoter compared to native YB-1, whereby it cannot be excluded that also a truncated YB-1 is migrating better into the nucleus and exhibits both activities, i.e. induces CPE and ninds to the E2-late promoter. Finally, such truncated YB-1 fragments can also migrate into the nucleus better and bind to the E2-late promoter better without inducing a better CPE. It is also
within the present invention that truncated YB-1 proteins or fragments comprise further sequences such as described herein in connection with the full length YB-1, in particular cellular localization signal sequences (NLS) and the like.

The adenoviruses used in accordance with the present invention are characterised by the various nucleic acids and gene products, respectively, disclosed herein and may otherwise comprise all those elements known to the ones skilled in the art and which are inherent to the wild type adenoviruses (Shenk, T.: Adenoviridae: The virus and their replication. Fields Virology, vol. 3, editors Fields, B.N., Knipe, D.M., Howley, P.M. et al., Lippincott-Raven Publishers, Philadelphia, 1996, chapter 67).

The invention is related in a further aspect to a method for the screening of patients which may be treated using the viruses in accordance with the present invention, whereby the method comprises the following steps:

- Analysing a sample of the tumor tissue and
- Determining whether YB-1 is localised in the nucleus of a cell, preferably a tumor stem cell independent of the cell cycle, or whether the cell contains deregulated/overexpressed YB-1 or whether the cells and preferably the tumor stem cells of the tumor express CD44.

In addition to YB-1 and CD44, respectively, also the presence of the afore-described markers can be assessed.

In case that the tumor tissue or a part thereof and in particular the tumor stem cells forming or contained in said tumor tissue comprises YB-1 in the nucleus, preferably independent of the cell cycle, or comprises deregulated YB-1, the adenoviruses as disclosed herein, may be used in accordance with the present invention.

In an embodiment of the method according to the invention it is contemplated that the analysis of the tumor tissue occurs by means of an agent which is selected from the group comprising antibodies against YB-1, aptamers against YB-1, spiegelmers against YB-1 as
well as anticalines against YB-1. In principle, the same kind of agents can also be made and used, respectively, for the respective markers. The manufacture of antibodies, in particular monoclonal antibodies, is known to the ones skilled in the art. A further agent for specific detection of YB-1 or the markers are peptides which bind with a high affinity to their target structures, in the present case YB-1 or said markers. In the prior art methods are known such as, for example, phage-display, in order to generate such peptides. For such purpose, it is started from a peptide library whereby the individual peptides have a length of about 8 to 20 amino acids and the size of the library is about $10^2$ to $10^{18}$, preferably $10^8$ to $10^{15}$ different peptides. A particular form of target molecule binding polypeptides are the so-called anticalines which are, for example, described in German patent application DE 197 42 706.

A further agent for specifically binding to YB-1 or the corresponding markers disclosed herein and thus for the detection of a cell cycle independent localisation of YB-1 in the nucleus, are the so-called aptamers, i.e. D-nucleic acids, which, based on RNA or DNA, are present as either a single strand or a double strand and specifically bind to a target molecule. The generation of aptamers is, for example, described in European patent EP 0 533 838. A special embodiment of aptamers are the so-called aptazymes which, for example, are described by Piganeau, N. et al. (2000), Angew. Chem. Int. Ed., 39, no. 29, pages 4369 - 4373. They are a particular embodiment of aptamers insofar as they comprise apart from the aptamer moiety a ribozyme moiety and, upon binding or release of the target molecule binding to the aptamer moiety, the ribozyme moiety becomes catalytically active and cleaves a nucleic acid substrate which goes along with generation of a signal.

A further form of the aptamers are the so-called spiegelmers, i.e. target molecule binding nucleic acids which consist of L-nucleic acids. The method for the generation of such spiegelmers is, for example, described in WO 98/08856.

The sample of the tumor tissue can be obtained by punctuation or surgery. The assessment whether YB-1 is located in the nucleus independent of the cell cycle is frequently done by the use of microscopic techniques and/or immunohistoanalysis, typically using the antibody or any of the further agents described above. Further methods for the detection of YB-1 in the nucleus and that its localisation there is independent of the cell cycle, are known to the one
skilled in the art. For example, localisation of YB-1 can easily be detected when scanning tissue slices stained against YB-1. The frequency of YB-1 being in the nucleus is already an indication that the localisation in the nucleus is independent of the cell cycle. A further possibility for cell cycle independent detection of YB-1 in the nucleus is the staining against YB-1 and assessment whether YB-1 is localised in the nucleus and determining the phase of the cells. This and the detection of YB-1, respectively, however, can also be performed using the afore-mentioned agents directed against YB-1. The detection of the agents is done by procedures known to the one skilled in the art. Because said agents are specifically directed against YB-1 and insofar do not bind to other structures within the sample to be analysed, particularly other structures of the cells, both the localisation of said agents by means of a suitable labelling of the agents and due to their specific binding to YB-1, also the localisation of YB-1 can be detected and assessed accordingly. Methods for the labelling of the agents are known to the ones skilled in the art.

The use of the adenoviruses in accordance with the present invention as medicaments and in particular in connection with systemic administration can be improved by a suitable targeting of the adenoviruses. The infection of tumor cells by adenovirus depends to a certain extent, among others, on the presence of the coxackievirus-adenovirus receptor CAR and particular integrins. If these are strongly expressed in cells, in particular tumor cells, an infection is already possible at very low titers (pfu/cell). Different strategies have so far been followed in order to achieve a so called re-targeting of the recombinant adenovirus by, for example, insertion of heterologous sequences in the fiber knob region and the C-terminus of protein IX, use of bi-specific antibodies, coating of the adenoviruses with polymers, introduction of ligands in the Ad-fibre, substitution of the serotype 5 knop and serotype 5 fiber shaft, respectively, and knop by the serotype 3 knop and Ad35 fiber shaft and knop and modification of the penton base (Nicklin S. A. et al., Molecular Therapy 2001, 4, 534-542; Magnusson, M. K. et al., J. of Virology 2001, 75, 7280-7289; Barnett B. G. et al., Biochimica et Biophysica Acta 2002, 1575, 1-14; Dimitrev IP et al., Journal of Virology, 2002, 76, 6893-6899; Mizuguchi und Hayakawa, Human Gene Therapy, 2004, 15, 1034-1044). Realizing such further designs and characteristics in connection with the adenoviruses in accordance with the present invention and the adenoviruses used in accordance with the present
invention, in particularly in connection with the adenoviruses of group I and/or group II in their various embodiments of the present invention, is within the present invention.

The various transgenes, including ElB55kD, E4orf6, ADP and the like, in particular if they are viral genes, may in principle be cloned from any respective virus, preferably adenovirus and more preferably adenovirus Ad5. A variety of plasmids are additionally described in the prior art which contain the respective genes and from which these may accordingly be taken and introduced into both the adenoviruses in accordance with the present invention as well as the viruses to be used in accordance with the present invention. An example for a plasmid expressing ElB55kD is, for example, described by Dobbelstein, M. et al., EMBO Journal, 16, 4276-4284, 1997. The coding region of the ElB55K gene can, for example, can be excised together with the 3’ non-coding region (the 3’UTR region lies preferably at about base position 3507 - 4107 of the adenovirus wild type genome) of this gene by means of Bam HI from the plasmid pDCREIB. The respective fragment comprising the ElB55kD gene as well as the 3’ non-coding region corresponds to nucleotides 2019 to 4107 of the adenovirus type 5. It is, however, also within the present invention that the ElB55kD gene is excised from the plasmid by means of the restriction enzymes Bam HI and Bfrl and Xbal, respectively, and subsequently cloned into the adenovirus. It is also within the present invention that also analogues thereof and in particular analogues of the 3’ UTR region may be used within the present invention. An analogue of the 3’ UTR region is any sequence which has the same effect as the 3’ UTR region, particularly the same effect with regard to the expression of a gene, preferably the ElB55kD gene. Such analogues can be determined by routine experiments performed by the ones skilled in the art, e.g. by extending or shortening the 3’ UTR region by one or several nucleotides and subsequently testing whether the thus obtained analogue still has the same effect as the 3’ UTR region as described previously. In an embodiment the term 3’ UTR region thus comprises also each and any analogue thereof.

Those viruses where therapeutic genes or transgenes are cloned in a preferred embodiment preferably under the control of a specific promoter, in particular a tumor-specific or tissue-specific promoter, are further developments of the viruses in accordance with the present invention. It is also within such viruses that also the E4 region is functionally inactive and is preferably deleted. The transgenes described herein can also be cloned into the E4 region,
whereby this may be performed alternatively or additionally to the cloning of the transgenes into the E3 region and the E3 region may remain partially or completely intact, respectively. Transgenes as used herein may be therapeutic genes or viral genes, preferably adenoviral genes, which preferably are not present in the genome of wild type adenoviruses and which are not present, respectively, at a site of the genome at which they are located in the particular virus now.

Therapeutic genes can be prodrug genes, genes for cytokines, apoptosis inducing genes, tumor suppressor genes, genes for metalloproteinase inhibitors and/or angiogenesis inhibitors, and tyrosine kinase inhibitors. Additionally, siRNA, aptamers, antisense molecules and ribozymes may be expressed which are preferably directed against cancer-relevant target molecules. Preferably the individual or the several target molecules are selected from the group comprising the resistance-relevant factors, anti-apoptosis factors, oncogenes, angiogenesis factors, DNA synthesis enzymes, DNA repair enzymes, growth factors and their receptors, transcription factors, metalloproteinases, particularly matrix metalloproteinases, and plasminogen activator of the urokinase type. Preferred embodiments thereof are already disclosed herein.

In an embodiment the resistance-relevant factors are preferably selected from the group comprising P-glycoprotein, MRP and GST and also comprise the nucleic acids coding therefor.

Possible prodrug genes as may be used in preferred embodiments, are, for example, cytosine deaminase, thymidine kinase, carboxypeptidase, uracil phosphoribosyl transferase; or purine nucleoside phosphorylase (PNP); Kirn et al, Trends in Molecular Medicine, volume 8, no. 4 (suppl), 2002; Wybranietz W.A. et al., Gene Therapy, 8, 1654-1664, 2001; Niculescu-Duvaz et al., Curr. Opin. Mol. Therapy, 1, 480.486, 1999; Koyama et al., Cancer Gene Therapy, 7, 1015-1022, 2000; Rogers et al., Human Gene Therapy, 7, 2235-2245, 1996; Lockett et al., Clinical Cancer Res., 3, 2075-2080, 1997; Vijayakrishna et al., J. Pharmacol. And Exp. Therapeutics, 304, 1280-1284, 2003.
Possible cytokines as may be used in preferred embodiments, are, for example, GM-CSF, TNF-alpha, 11-12, 11-2, 11-6, CSF or interferon-gamma; Gene Therapy, Advances in Pharmacology, volume 40, editor: J. Thomas August, Academic Press; Zhang and Degroot, Endocrinology, 144, 1393-1398, 2003; Descamps et al., J. Mol. Med., 74, 183-189, 1996; Majumdar et al., Cancer Gene Therapy, 7, 1086-1099, 2000.

In an embodiment the anti-apoptosis factors are selected from the group comprising BCL2 and comprise also the nucleic acids coding therefor. In an embodiment the oncogenes are selected from the group comprising Ras, particularly mutated Ras, Rb and Myc, and comprises also the nucleic acids coding therefor. In an embodiment the angiogenesis factors are selected from the group comprising VEGF and HMG proteins, and also comprise the nucleic acids coding therefor. In an embodiment the DNA synthesis enzymes are selected from the group comprising telomerase, and also comprise the nucleic acids coding therefor. In an embodiment the DNA repair enzymes are selected from the group comprising Ku-80, and also comprise the nucleic acids coding therefor. In an embodiment the growth factors are selected from the group comprising PDGF, EGF and M-CSF, and also comprise the nucleic acids coding therefor. In a further embodiment the receptors are in particular those of growth factors, whereby preferably the growth factors are selected from the group comprising PDGF, EGF and M-CSF, and also comprise the nucleic acids coding therefor. In an embodiment the transcription factor is selected from the group comprising YB-1, and also comprises the nucleic acid coding therefor. In an embodiment the metalloproteinases are in particular matrix metalloproteinases. In a preferred embodiment the matrix metalloproteinases are selected from the group comprising MMP-1 and MMP-2, and also comprise the nucleic acids coding therefor. In an embodiment the plasminogen activators of the urokinase type are selected from the group comprising uPa-R, and also comprise the nucleic acids coding therefor.


Possible tumor suppressor genes as may be used in preferred embodiments, are, for example, ElA, p53, p16, p21, p27 or MDA-7: Opalka et al., Cell Tissues Organs, 172, 126-132, 2002, Ji et al., Cancer Res., 59, 3333-3339, 1999, Su et al., Oncogene, 22, 1164-1180, 2003.

Possible angiogenesis inhibitors as may be used in preferred embodiments, are, for example, endostatin or angiostatin: Hajitou et al., FASEB J., 16, 1802-1804, 2002, and antibodies against VEGF: Ferrara, N., Semin Oncol 2002 Dec; 29 (6 suppl 16): 10-4.

Possible metalloproteinase inhibitors as may be used in preferred embodiments, are, for example, Timp-3 [Ahonen et al., Mol Therapy, 5, 705-715, 2002]; PAI-1 [Soff et al., J. Clin. Invest., 96, 2593-2600, 1995]; Timp-1 [Brandt K. Curr. Gene Therapy, 2, 255-271, 2002].

Further transgenes in the sense of the present invention which may be expressed by both group I adenoviruses and group II adenoviruses in accordance with the present invention are also tyrosine kinase inhibitors. Exemplary tyrosine kinases are EGFR (epidermal growth factor receptor) [Onkologie, Entstehung und Progression maligner Tumoren; author: Christoph Wagner, Georg Thieme Verlag, Stuttgart, 1999]. A preferred tyrosine kinase inhibitor is herceptin [Zhang H et al., Cancer Biol Ther. 2003, Jul-Aug; 2 (4 suppl 1): S122-6].

SiRNA (short interfering RNA), as may be used within the present invention, consists of two, preferably separate RNA strands which hybridise to each other due to base complementarity which means that they are present essentially base paired and preferably have a length of up to 50 nucleotides, preferably between 18 and 30 nucleotides, more preferably less than 25 nucleotides and most preferably 21, 22 or 23 nucleotides, whereby these figures refer to the single strand of the siRNA, particularly to the length of the stretch of the single strand which hybridises to or is base paired with a, more precisely the second single strand. siRNA specifically induces or mediates the degradation of mRNA. The specificity required
theretofore is mediated by the sequence of the siRNA and thus its binding site. The target sequence to be degraded is essentially complementary to the first or to the second of the siRNA forming strands. Although the precise mode of action is not yet clear, it is assumed that siRNA is a biological strategy for cells in order to inhibit distinct alleles during development and to protect themselves against viruses. siRNA mediated RNA interference is used as a method for the specific suppression or complete elimination of the expression of a protein by introducing a gene specific double-stranded RNA. For higher organisms a siRNA comprising 19 to 23 nucleotides is insofar particularly suitable as it does not result in the activation of a non-specific defense reaction such as an interleukin response. The direct transfection of double-stranded RNA of 21 nucleotides having symmetrical 2-nucleotide 3' overhangs was suitable to mediate RNA interference in mammalian cells and is highly efficient compared to other technologies such as ribozymes and antisense molecules (Elbashir, S. Harborth J. Lendeckel W. Yalvcin, A. Weber K, Tuschl T: Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature 2001, 411: 494-498). As little as a few siRNA molecules are sufficient so as to suppress expression of the target gene. In order to avoid the limitations of exogenously added siRNA which particularly reside in the transient nature of the interference phenomenon and specific delivery (delivery) of the siRNA molecules, vectors are used in the prior art which allow for an endogenous siRNA expression. For such purpose, for example, oligonucleotides having a length of 64 nucleotides are introduced into the vector which comprise the 19 nucleotide long target sequence both in the sense and in the antisense orientation, separated by, for example, a 9 nucleotide spacer sequence. The resulting transcript folds into a hairpin structure with a stem structure (stem) of, for example, 19 base pairs. The loop is rapidly degraded in the cell so that a functional siRNA molecule is generated (Brummelkamp et al., Science, 296, 550-553, 2002).

In a still further embodiment the medicament further comprises at least one pharmaceutically active compound.

In a preferred embodiment the pharmaceutically active compound is selected from the group comprising cytokines, metalloproteinase inhibitors, angiogenesis inhibitors, cytostatics such as Irinotecan and CPT-11 against colorectal carcinoma and Daunorubicin against leukemia, cell cycle inhibitors such as CYC202 which inhibits CDK2/CyclinE kinase activity and can
be used against colorectal tumors (McClue SJ, Int. J. Cancer 2002, 102, 463-468) and BAY 43-9006 which inhibits Raf-1 and is, for example, effective against mamma carcinoma (Wilhelm SM et al., Cancer Res. 2004, 64, 7099-7109), proteosome inhibitors such as PS-341 which inhibits the 26S proteasome activity and is used against squamous-cell carcinoma (Fribley A et al., Mol Cell Biol 2004 Nov; 24(22): 9695-704), recombinant antibodies such as against the EGF receptor (Herceptin for breast carcinoma and prostate tumor; H.G. van der Poel, European Urology 2004, 1-17; Erbitux against head and neck tumors; Bauman M et al., Radiother. Oncol., 2004, 72, 257-266), and inhibitors of the signal transduction cascade such as STI 571 which represses, among others, c-kit and can be used against gastrointestinal tumors (H.G. van der Poel, European Urology 2004, 45, 1-17), ABT-627 an endothelin inhibitor which may be used, among others, against prostate tumors (H.G. van der Poel, European Urology 2004, 45, 1-17), SU5416 which inhibits phosphorylation of the VEGF tyrosine kinase receptor and which may be used against glioblastoma and prostate cancer (Bischof M et al Int. J. Radiat. Oncol. Biol. Phys. 2004; 60 (4): 1220-32), ZD1839 which inhibits EGFR tyrosine activity and may be used, among others, against prostate tumors (H.G. van der Poel, European Urology 2004, 45, 1-17); rapamycin derivatives such as CCI-779 and RAD001 which inhibit mTOR and can be used against prostate tumors. It is within the present invention that the various adenoviruses described herein and the adenoviruses to be used in accordance with the present invention, respectively, can, in principle, be used with each and any of the aforementioned compounds for each and any of the indication described in connection therewith. In a particularly preferred embodiment the indication is the one which is described for any of the previously mentioned pharmaceutically active compounds.

The present inventor has furthermore surprisingly found that the efficacy of the viruses described herein and in particular the viruses used in accordance with the present invention can be increased by using in combination at least two compound whereby each of the at least two compounds is individually and independently selected from the group comprising cytostatics.

As used herein in a preferred embodiment, cytostatics are in particular chemical or biological compounds which, during or after the administration to a cell or an organism containing a or such cell, result in the cell no longer growing and/or no longer dividing or slowing down cell
division and/or cell growth. Cytostatics also comprise compounds which turn into a cytostatic in the aforedescribed sense only in the cell or in an organism containing such cell. Insofar, the term cytostatics also comprises pre-cytostatics.

Cytostatics are grouped according to their mode of action. The following groups are distinguished which, in principle, can all be used within the present invention:

- Alkylating agents, i.e. chemical compounds which cause their cytotoxic effect by alkylating phosphate, amino, sulphhydryl, carboxy and hydroxy groups of the nucleic acid as well as proteins. Such compounds are often cancerogenic themselves. Typical examples of this group of cytostatics are cis-platin and platin derivatives, cyclophosphamide, dacarbazine, mitomycin, procarbazine.

- Antimetabolites, i.e. compounds which, due to their structural similarity or ability for binding block a metabolic process or affect the same. Within the group of antimetabolites it is distinguished between structurally similar antimetabolites, structure changing antimetabolites and the indirectly acting antimetabolites. The structurally similar antimetabolites compete due to chemical similarity with the metabolite without exerting the function thereof. Structure changing antimetabolites bind to the metabolites which impedes its function or resorption or chemically modifies the metabolite. Indirectly acting antimetabolites interfere with the function of the metabolite, for example by the binding of ions. Typical examples of this group are folic acid antagonists such as methotrexate, pyrimidine analogues such as fluorouracil, purine analogues such as azathioprine and mercaptopurine.

- Mitosis inhibitors, i.e. compounds which inhibit cell division. Within the group of mitosis inhibitors it is distinguished between cell division toxins, spindle toxins and chromosome toxins. Typical examples of this group are taxanes and vinca alkaloids. The taxanes in turn can be divided into the two major groups of taxoles and taxoters, whereby a particularly preferred taxole is paclitaxel, and a particularly preferred taxoter is docetaxel.
Antibiotics having an inhibitory effect on the DNA-dependent RNA polymerase. Typical examples are the anthracyclines, such as, e.g., bleomycin, daunorubicin, doxorubicin and mitomycin.

Topoisomerase inhibitors, in particular topoisomerase I inhibitors. Topoisomerase inhibitors are chemical compounds which determine the tertiary structure of the DNA by catalysing the change of the DNA twist number in a three stage process. Essentially, two forms of topoisomerases are distinguished. Topoisomerases of type I cleave only a DNA strand and are ATP-independent, whereas topoisomerase of type II cleave both strands of a DNA, whereby they are ATP-dependent. Typical examples for topoisomerase I inhibitors are irinotecan and topotecan, and for topoisomersae II inhibitors etoposid and daunorubicin.

Within the present invention at least one and preferably two agents are selected from the aforementioned group. It is, however, also within the invention that in particular also three, four or five different agents are selected. The following comments are made for the embodiment of the present invention where only one and preferably two agents are used together with the virus. These considerations are basically also applicable to the embodiments where more than two agents are used.

Preferably, the agents differ from each other such that they address different target molecules or are described in literature as targeting different molecules. It is within the present invention that the agent also comprises two or more different agent which bind to the same target molecule. It is also within the present invention that one agent binds to a first site of the target molecule, whereas the second agent binds to a second site of the target molecule.

It is also within the present invention that at least two of the agents are active using different modes of action. Active means in a preferred embodiment that the cell growth and/or cell division inhibiting or retarding effect of the chemical compound is mediated through a different mode of action. In a particularly preferred embodiment the term active means that the replication efficiency of a virus, in particular the virus in accordance with the present invention, of the viruses described herein and of the viruses to be used in accordance with the present invention, is increased compared to a scenario where one and/or both of the agents are
not used. As a measure for the efficiency of viral replication preferably the number of viruses required for cell lysis is used, preferably expressed as pfu/cell.

In a particularly preferred embodiment at least one of the at least two agents is one which increases the infectability of the cell in which the replication of the virus is to occur, preferably is to occur in a selective manner, preferably with the virus described herein and/or the virus to be used in accordance with the present invention. This can, e.g., be performed by increasing the uptake of the virus by the cell. The uptake of the virus, in particular of adenovirus, is, for example, mediated by the coxsackievirus-adenovirus receptor (CAR) (Mizuguchi und Hayakawa, GENE 285, 69-77, 2002). An increased expression of CAR is, for example, caused by trichostatin A (Vigushin et al., Clinical Cancer Research, 7, 971-976, 2001).

In a further embodiment one of the at least two agents is one which increases the availability of a component within the cell, whereby the component is one which increases the replication of the virus, preferably the virus described herein and/or the virus to be used in accordance with the present invention.

In a further embodiment one of the at least two agents is one which mediates the transport of YB-1 into the nucleus. Such an agent can be selected from the group comprising topoisomerase inhibitors, alkylating agents, antimetabolites and mitosis inhibitors. Preferred topoisomerase inhibitors are camptothecin, irinotecan, etoposide and their respective analogues. Preferred mitosis inhibitors are daunorubicin, doxorubicin, paclitaxel and docetaxel. Preferred alkylating agents are cis-platin and their analogues. Preferred antimetabolites are fluorouracil and methotrexat.

In a particularly preferred embodiment one of the at least two agents is one which increases the infectability of the cell, in particular the expression of CAR, and the second of the at least agents is one which increases the transport of YB-1 into the nucleus, whereby preferably as chemical compound a compound is used which exhibits the respective required characteristic as preferably described above.
In a further embodiment the one of the at least two agents is a histone deacylase inhibitor. A preferred histone deacylase inhibitor is one which is selected from the group comprising trichostatin A, FR901228, MS-27-275, NVP-LAQ824 and PXDIOI. Trichostatin A is, for example, described in Vigushin et al., Clinical Cancer Research, 7, 971-976, 2001; FR901228 is, for example, described in Kitazono et al., Cancer Res., 61, 6328-6330, 2001; MS-27-275 is described in Jaboin et al., Cancer Res., 62, 6108-6115, 2002; PXDIOI is described in Plumb et al., Mol. Cancer Ther., 8, 721-728, 2003; NVP-LAQ824 is described in Atadja et al., Cancer Res., 64, 689-695, 2004.

In an embodiment at least one agent is selected from the group comprising trichostatin A (against glioblastoma, Kim JH et al., Int. J. Radiation Oncology Biol. Phys. 2004, 59, 1174-1180), FR 901228 (against pancreas tumors, Sato N et al., Int. J. Oncol. 2004, 24, 679-685; MS-27-275 (against prostate tumors; Camphausen K et al., Clinical Cancer Research 2004, 10, 6066-6071), NVP-LAQ824 (against leukemia; Nimmanapalli R et al., Cancer Res. 2003, 63, 5126-5135; PXDIOI (against ovary tumors, Plumb JA et al, Mol. Cancer Ther. 2003, 2, 721-728), scriptaid (against breast carcinoma, Keen JC et al., Breast Cancer Res. Treat. 2003, 81, 177-186), apicidin (against melanoma, Kim SH et al., Biochem. Biophys. Res. Commun. 2004, 315, 964-970) and CI-994 (against various tumors, Nemunaitis JJ et al., Cancer J. 2003, 9, 58-66). The mode of action of histone deacetylase inhibitors is, among others, described in Lindemann RK et al., Cell Cycle 2004, 3, 77-86. It is within the present invention that the various adenoviruses described herein and the adenoviruses to be used in accordance with the present invention, may be used with the aforementioned compounds, in principle, for each and any of the indications described herein in connection therewith. In a particularly preferred embodiment the indication is one as has been described for each and any of the aforementioned pharmaceutically active compounds.

In a still further embodiment the one of the at least two agents is a topoisomerase inhibitor, preferably a topoisomerase I inhibitor. A preferred topoisomerase inhibitor is one which is selected from the group comprising camptothecin, irinotecan, topotecan, SN-38, 9-aminocamptothecin, 9-nitrocamptothecin, DX-895If and daunorubicin. Irinotecan and SN-38 are, for example, described in Gilbert et al., Clinical Cancer Res., 9, 2940-2949, 2003; DX-895IF is described in van Hattum et al., British Journal of Cancer, 87, 665-672, 2002;

In a preferred embodiment the topoisomerase inhibitor is selected from the group comprising camptothecin, irinotecan, topotecan, DX-895If, SN-38, 9-aminocamptothecin, 9-nitrocamptothecin, etoposide and daunorubicin. These may be used against various tumors, for example, colorectal tumors, pancreas tumors, ovary carcinomas and prostate carcinomas. The fields of application are, among others, described by Recchia F et al., British J. Cancer 2004, 91, 1442-1446; Cantore M et al., Oncology 2004, 67, 93-97; Maurel J. et al., Gynecol. Oncol. 2004, 95, 114-119; Amin A. et al., Urol. Oncol. 2004, 22, 398-403; Kindler HL et al., Invest. New Drugs 2004, 22, 323-327, Ahmad T. et al., Expert Opin. Pharmacother. 2004, 5, 2333-2340; Azzariti A. et al., Biochem Pharmacol. 2004, 68, 135-144; Le QT et al., Clinical Cancer Res. 2004, 10, 5418-5424. It is within the present invention that the various adenoviruses described herein and the adenoviruses to be used in accordance with the present invention, respectively, may in principle be used with the aforementioned compounds for each and any of the indications described herein in connection therewith. In a particularly preferred embodiment the indication is such as described for each of the aforementioned pharmaceutically active compounds.

In an embodiment at least one agent is selected from the group comprising trichostatin A, FR 901228 (against pancreas tumors, Sato N et al., Int. J. Oncol. 2004, 24, 679-685; MS-27-275 (against prostate tumors; Camphausen K et al., Clinical Canver Research 2004, 10, 6066-6071), NVP-LAQ824 (against leukemias; Nimmanapalli R et al., Cancer Res. 2003, 63, 5126-5135; PXD101 (against ovary tumors, Plumb JA et al, Mol. Cancer Ther. 2003, 2, 721-728) scriptaid (against breast carcinoma, Keen JC et al., Breast Cancer Res. Treat. 2003, 81, 177-186), apicidin (against melanoma, Kim SH et al., Biochem. Biophys. Res. Commun. 2004, 315, 964-970) and CI-994 (against various tumors, Nemunaitis JJ et al., Cancer J. 2003, 9, 58-66). The mode of action of histone deacetylase inhibitors is, among others, described in Lindemann RK et al., Cell Cycle 2004, 3, 77-86. It is within the present invention that the various adenoviruses described herein and the adenoviruses to be used in accordance with the
present invention, may be used with the aforementioned compounds, in principle, for each and any of the indications described herein in connection therewith. In a particularly preferred embodiment the indication is one as has been described for each and any of the aforementioned pharmaceutically active compounds.

In a preferred embodiment the topoisomerase inhibitor is selected from the group comprising camptothecin, irinotecan, topotecan, DX-895If, SN-38, 9-aminocamptothecin, 9-nitrocamptothecin, daunorubicin and etoposid. These may be used against various tumors, for example, colorectal tumors, pancreas tumors, ovary carcinomas, lung tumors and prostate carcinomas. The fields of application are, among others, described by Recchia F et al., British J. Cancer 2004, 91, 1442-1446; Cantore M et al., Oncology 2004, 67, 93-97; Maurel J. et al., Gynecol. Oncol 2004, 95, 114-119; Amin A. et al., Urol. Oncol. 2004, 22, 398-403; Kindler HL et al., Invest. New Drugs 2004, 22, 323-327, Ahmad T. et al., Expert Opin. Pharmacother. 2004, 5, 2333-2340; Azzariti A. et al., Biochem Pharmacol. 2004, 68, 135-144; Le QT et al., Clinical Cancer Res. 2004, 10, 5418-5424. It is within the present invention that the various adenoviruses described herein and the adenoviruses to be used in accordance with the present invention, respectively, may in principle be used with the aforementioned compounds for each and any of the indications described herein in connection therewith. In a particularly preferred embodiment the indication is such as described for each of the aforementioned pharmaceutically active compounds.

In a particularly preferred embodiment the one of the at least two agents is a histone deacetylase inhibitor and the other one of the at least two agents is a topoisomerase inhibitor.

In a preferred embodiment of each and any aspect of the present invention the further pharmaceutically active compound is selected from the group comprising cytokines, metalloproteinase inhibitors, angiogenesis inhibitors, cytostatics such as irinotecan and CPT-11 against colorectal carcinoma and daunorubicin against leukemia, cell cycle inhibitors such as CYC202 which inhibits CDK2/CyclinE kinase activity and can be used against colorectal tumors (McClue SJ, Int. J. Cancer 2002, 102, 463-468) and BAY 43-9006 which inhibits Raf-1 and is effective against mamma carcinoma (Wilhelm SM et al., Cancer Res. 2004, 64, 7099-7109), proteosome inhibitors such as PS-341 which inhibits the 26S proteasome activity and
is used against brain tumors (Yin D. et al., Oncogene 2004), recombinant antibodies such as against the EGF receptor (Herceptin for breast carcinoma and prostate tumor; H.G. van der Poel, European Urology 2004, 1-17; Erbitux against head and neck tumors; Bauman M et al., Radiother. Oncol., 2004, 72, 257-266), and inhibitors of the signal transduction cascade such as STI 571 which represses, among others, c-kit and can be used against gastrointestinal tumors (H.G. van der Poel, European Urology 2004, 45, 1-17), ABT-627 an endothelin inhibitor which may be used, among others, against prostate tumors (H.G. van der Poel, European Urology 2004, 45, 1-17), SU5416 which inhibits phosphorylation of the VEGF tyrosine kinase receptor and which may be used against head/neck tumors (Cooney et al., Cancer Chemother. Pharmacol 2004), ZD1839 which inhibits EGFR tyrosine activity and may be used, among others, against prostate tumors (H.G. van der Poel, European Urology 2004, 45, 1-17); rapamycin derivatives such as CCI-779 and RADOOI which inhibit mTOR and can be used against prostate tumors (H.G. van der Poel, European Urology 2004, 45, 1-17). It is within the present invention that the various adenoviruses described herein and the adenoviruses to be used in accordance with the present invention, respectively, can, in principle, be used with each and any of the aforementioned compounds for each and any of the indications described in connection therewith. In a particularly preferred embodiment the indication is the one which is described for any of the previously mentioned pharmaceutically active compounds.

In an embodiment the means according to the present invention and/or the means prepared in accordance with the present invention contains the virus separate from one or several of the at least one and preferably at least two agents which are combined with the virus in accordance with the present invention. It is preferred that the virus is separate from any agent which is combined with the virus. Preferably the separation is a spatial separation. The spatial separation can be such that the virus is present in a different package than the agent. Preferably the package is a single dose unit, i. e. the virus and the agent are packed as single dosages. The single dose units may in turn be combined to form a package. However, it is also within the present invention that the single dosages of the virus are combined with one or several single dosages of one or several of the agents or packed therewith.
The kind of package depends on the way of administration as known to the one skilled in the art. Preferably the virus will be present in a lyophilized form or in a suitable liquid phase. Preferably, the agents will be present in solid form, e. g. as tablets or capsules, however, are not limited thereto. Alternatively, also the agents can be present in liquid form.

It is within the present invention that the virus is systemically or locally administered. It is also within the present invention that the agents combined with the virus are systemically or locally administered individually and independently from each other or together. Other modes of administration are known to the ones skilled in the art.

It is within the present invention that the virus and the agents combined with it, are administered in a chronologically separate manner or at the same time. In connection with a chronologically separate manner it is preferred that the agent is administered prior to the administration of the virus. How long the agent is administered prior to the virus depends on the kind of the agent used and is obvious for the one skilled in the art from the mode of action of the agent used. Also the administration of the at least two agents can occur at the same or at different points in time. In connection with a chronologically different administration the points of time again result from the modes of action underlying the agents and can, based thereon, be determined by the ones skilled in the art.

The above considerations, given in connection with the medicaments according to the present invention which are also referred to herein as pharmaceutical compositions, are roughly also applicable to any composition, including compositions as used for the replication of viruses, preferably for the in vitro replication of viruses in accordance with the present invention. The above considerations are also applicable to the kit in accordance with the present invention and the kit to be used in accordance with the present invention, respectively, which may apart from the viruses described herein and the viruses to be used in accordance with the invention, also comprise an agent or a combination of agents as described herein. Such kits comprise the virus and/or the one or the several agents in a form ready for use and preferably instructions for use. Furthermore, the above embodiments apply also to the nucleic acids as disclosed herein, and the nucleic acids used in accordance with the present invention, and the replication systems in accordance with the present invention and the nucleic acids coding
therefor, and the replication systems used in accordance with the present invention and the nucleic acids coding therefor used in accordance with the present invention.

The medicament in connection with which or for the manufacture of which the adenoviruses disclosed herein are used in accordance with the present invention, is intended to be applied, usually, in a systemic manner, although it is also within the present invention to apply or deliver it locally. The application is intended to infect particularly those cells with adenoviruses and it is intended that adenoviral replication particularly occurs therein, which are involved, preferably in a causal manner, in the formation of a condition, typically a disease, for the diagnosis and/or prevention and/or treatment of which the inventive medicament is used.

Such a medicament is preferably for the treatment of tumor diseases. Those tumor diseases are particularly preferred where where YB-1 is, due to the mechanism underlying the tumor disease, in particular due to the underlying pathological mechanism, already located in the nucleus, or where the presence of YB-1 in the cellular nucleus is caused by exogenous measures whereby such exogenous measures are suitable to transfer YB-1 into the cellular nucleus or to induce or to express it there. The term tumor or tumor disease shall comprise herein both malignant as well as benign tumors, and respective diseases. In an embodiment the medicament comprises at least one further pharmaceutically active compound. The nature and the amount of such further pharmaceutically active compound will depend on the kind of indication for which the medicament is used. In case the medicament is used for the treatment and/or prevention of tumor diseases, typically cytostatics such as cis-platin and taxole, daunoblastin, daunorubicin, adriamycin and/or mitoxantrone or others of the cytostatics or groups of cytostatics described herein.

The medicament in accordance with the invention can be present in various formulations, preferably in a liquid form. Furthermore, the medicament will contain adjuvants such as stabilisers, buffers, preservatives and the like which are known to the one skilled in the art of formulations.
The medicament in connection with which or in connection with the manufacture of which the adenoviruses described herein are used in accordance with the present invention is envisaged to be typically administered in a systemic manner, although it is also within the present invention that it is applied locally or delivered locally. The application intends to infect those cells with the adenovirus and to cause adenoviral replication therein, which are involved, preferably in a causal manner, in the formation of a condition, typically a disease for the diagnosis and/or prevention and/or treatment of which the medicament according to the present invention is used.

Such a medicament is preferably for the treatment of tumor diseases. Those tumor diseases are particularly preferred where where YB-1 is, due to the mechanism underlying the tumor disease, in particular due to the underlying pathological mechanism, already located in the nucleus, or where the presence of YB-1 in the cellular nucleus is caused by exogenous measures whereby such exogenous measures are suitable to transfer YB-1 into the cellular nucleus or to induce or to express it there. The term tumor or tumor disease shall comprise herein both malignant as well as benign tumors, and respective diseases. In an embodiment the medicament comprises at least one further pharmaceutically active compound. The nature and the amount of such further pharmaceutically active compound will depend on the kind of indication for which the medicament is used. In case the medicament is used for the treatment and/or prevention of tumor diseases, typically cytostatics such as cis-platin and taxole, daunoblastin, daunorubicin, adriamycin and/or mitoxantrone or others of the cytostatics or groups of cytostatics described herein.

The medicament in accordance with the invention can be present in various formulations, preferably in a liquid form. Furthermore, the medicament will contain adjuvants such as stabilisers, buffers, preservatives and the like which are known to the one skilled in the art of formulations.

It will be appreciated that any feature disclosed in connection with a specific aspect of the present invention is also a feature of any one of the other aspects of the present invention. Accordingly, what has been disclosed in connection with the various viruses applies equally
to the method using such viruses or pharmaceutical compositions and medicaments, respectively, and vice versa.

The invention is now further illustrated by the following figures and examples.

Fig. 1 is an illustration of the result of a Western blot analysis detecting YB-1 in glioma cells U87 and U373, in tumor stem cells R11, R28 and R49.

Fig. 2 are light microscopic images showing the cytopathic effect of an adenovirus which replicates in a YB-dependent manner, temozoloide, an E1 minus adenovirus, wild type adenovirus and a control on glioma derived tumor stem cells R11, R28, R40 and R49.

Fig. 3 is a Kaplan-Meier curve showing the survival rate of mice after implantation of glioma stem cells into the brain using various treatments regimens, namely adenovirus delo3RGD alone, adenovirus delo3RGD together with temozolomide, temozolomide alone and phosphate buffered saline (PBS).

Fig. 4 is a diagram depicting the relative replication of a YB-dependent adenovirus in glioma cells U373 under normoxia and hypoxia conditions.

Fig. 5 is a diagram depicting the relative replication of a YB-dependent adenovirus in glioma cells U87 under normoxia and hypoxia conditions.

Fig. 6 is a diagram depicting the relative replication of a YB-dependent adenovirus in glioma derived tumor stem cells R40 under normoxia and hypoxia conditions.

Fig. 7 shows the result of a clonogenic dilution assay using R11, R28 and R40 as tumor stem cells which were cultivated either under normoxia or hypoxia in the presence of TMZ, AdEl-minus, Ad-Delo3RGD, wild type adenovirus and a control, respectively at different cell dilutions.
Fig. 8 is a diagram depicting the fold increase of adenoviral fiber copies for tumor stem cells R11, R28 and R40 upon replication of dl703, Ad-Delo3RGD (delo) and wild type adenovirus.

Fig. 9 shows the result of a Western blot analysis indicating that Ad-Delo3RGD causes downregulation of CD44 expression in R28 tumor stem cells.

**Example 1: Material and Methods**

The following materials and methods were used in connection with the examples contained herein if not indicated differently.

**Adenoviral vectors**

The oncolytic adenovirus Ad-Delo3-RGD (Ad-DeloRGD) which is also referred to herein as Delo3RGD, Delo3-RGD or delo, has been published by Rognoni et al. (Adenovirus-based virotherapy enabled by cellular YB-1 expression in vitro and in vivo. Cancer Gene Ther 2009;16:753-63). The El-minus adenovirus dl70-3 has been published by Bett et al. (An efficient and flexible system for construction of adenovirus vectors with insertions or deletions in early regions 1 and 3. Proc Natl Acad Sci USA 1994;91 :8802-6). Ad-Delo3-RGD, dl703 and Ad-WT of serotype 5 were produced in HEK293 cells and purified by two consecutive standard cesium chloride gradient centrifugations and size-exclusion chromatography (Disposable PD-10 Desalting Columns, GE Healthcare, Munich, Germany).

Viral titers were determined by plaque assay using HEK293 cells.Multiplicity of infection (MOI) is therefore indicated as plaque forming units (pfu) per cell and virus dose was optimized for each *in vitro* experiment.

Adenovirus dl703: the E1 deletion is related to the Sspl restriction site (AATAAT) nt 339 and AflIII (CTTAAG) nt 3533. The deletion in dl703 is also referred to as 3.2 -kb deletion (Bett et al 1994, supra).

Adenovirus AdEGFPRGD: comprises the following El-Deletion: the E1 deletion is related to the Sspl restriction site (AATAAT) nt 339 and AflIII (CTTAAG) nt 3533; accordingly, the deletion is identical to the one of dl703.

**Cytopathic effect (CPE) assay**

To verify the effect of AdDelo3RGD on glioma stem cells a so-called cytopathic effect assay were performed. Brain Tumor Initiating Cells (BTIC: R11, R28, R40) were obtained from patients with primary glioblastoma as previously described (Beier D, Hau P, Proescholdt M et al. CD133+ and CD133- glioblastoma derived cancer stem cells show differential growth characteristics and molecular profiles. Cancer Research 2007; 67: 4010-4015) and were maintained as neurospheres in stem cell-permissive DMEM-F12 medium supplemented with 20 ng/mL of each human recombinant epidermal growth factor, human recombinant basic fibroblast growth factor (R & D Systems, Minneapolis, MN), human leukemia inhibitory factor (Chemicon, Schwalbach, Germany), and 2% B27 (B-27 serum free supplement (50x), liquid; Invitrogen, Cat: 17504-044). R11, R28 and R40 cells were seeded in 12-well plates (1 x 10^5 cells in 0.5 ml stem cell medium per well) and infected the next day with indicated virus or treated with TMZ. AdDelo3RGD or E1-minus adenovirus dl703 (50 pfu) (Bert AJ et al., Proc Natl Acad Sci USA 1994, 91, 8802-6.) Temozolomide (TMZ) treatment was performed by adding TMZ to the media in a final concentration of 100 µM. 5-7 days later cells were examined by lightmicroscopy.

**Hypoxia and viral replication**

U373, U87, and R11 (tumor stem cells) cells were seeded in 12-well plates (1 x 10^5 per well) and infected the next day with 20 PFU of Ad-WT or AdDelo3RGD. Twenty-four hours later cells were cultivated for further 24-36 h under normoxic or hypoxic conditions. After 48-60 h of infection cells was collected, DNA isolated by phenol/chloroform extraction and expression of virus hexon DNA and host β-actin DNA was analyzed by real-time PCR. Immunhistochemical staining of YB-1 revealed over-expression of YB-1 which is partly present in the nucleus.
Quantitative real-time PCR
Quantitative viral replication analysis was done with the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA) using a SYBR-green fluorescent dye (Agilent Technologies, Boeblingen, Germany) and the specific primers hexon-fw: 5'-GGC-CAT-TAC-CTT-TGA-CTC-TTC-3' (SEQ ID NO: 1), hexon-rev: 5'-GCA-TTT-GTA-CCA-GGA-ACC-AGT-C-3' (SEQ ID NO: 2), β-actin-fw: 5'-TAA-GTA-GGT-GCA-CAG-TAG-GTC-TGA-3' (SEQ ID NO: 3), and β-actin-rev: 5'-AAA-GTG-C AA-AGA-ACA-CGG-CTA-AG-3' (SEQ ID NO: 4). Cycling conditions started with initial enzyme activation at 95 °C for 15 min, followed by 40 cycles of 15 sec denaturation at 95 °C, 15 sec annealing at 60 °C, and 15 sec elongation at 72 °C. Homogeneity of amplification product was confirmed by melting curve analysis (T_m-hexon: 85 °C, T_m-B-actin: 83 °C). The amount of template DNA was 100 ng, 2 pmol of the forward and reverse primer Total in a total volume of 20 μl.

Animal experiments
Orthotopic mouse model were established in 6-week-old NMRI-nu (nu/nu) mice (Janvier, France) by injecting 1 x 10^5 R28 cells in 2 μL PBS directly into the brain (1 μL per minute). Mice were randomized into four groups (n = 7-8). The first group received 5 mg/kg TMZ in 2 μL on days 10 and 17 post infection. The second group received PBS. The third group received on day 7 post infection 3 x 10^8 PFU in 2 μl AdDelo3RGD. The last group received a treatment combination of virus and TMZ at the above mentioned days.

Western Blot Analysis
Cell were lysed with the lysis-buffer ProteoJET Mammalian Cell Lysis Reagent from Fermentas (USA) supplemented with complete protease inhibitor cocktail (Roche) and incubated at room temperature for 10 minutes. The lysates were clarified by centrifugation.
Protein concentrations were determined using the BCA Protein Assay kit (Thermo, USA). Protein (40 μg) was electrophoresed on 10% SDS polyacrylamide gels and electro-blotted onto Hybond-ECL nitrocellulose membrane (Amersham,USA). Alternatively, a polyvinylidenedifluoride membrane (Millipore) may be used. The membrane was incubated overnight at 4°C in 5 % BSA in PBS, washed with PBS/Tween then probed at RT with the YB-1- directed antibody (Abeam, USA, Dilution 1:300). After washing of the membrane the
membrane was incubated with the secondary horseradish peroxidase (HRP)-conjugated polyclonal antibody (Dako, Denmark Dilution 1: 1000). The bands were visualized using the image-station from Kodak. Alternatively, YB-1 and actin can be detected by using anti-actin (20-33) from SIGMA and an antibody which is specific for the N-terminus of YB-1 (Holzmuller et al. INT J CANCER 2011; 129: 1265-76). Immunoreactive proteins can be detected by using the enhanced chemiluminescence (ECL) or ECL plus western blot detection system (Amersham, Germany)

Cell lines and tissues
R11, R28, R49: Brain Tumor Initiating Cells to which it is also referred to herein as brain tumor stem cells or glioma derived tumor stem cells
U87, U373: glioblastoma derived cell lines
Normal Brain Tissue was obtained from a patient without any sign of cancer

Example 2: Detection of YB-1 in glioma cells and glioma derived tumor stem cells

YB-1 expression was analysed in glioma cell lines U87 and U373, and in tumor stem cells R11, R28 and R49 (Lottaz C, Cancer Res; 70(5) March 1, 2010; published online first on February 9, 2010). Normal brain tissue, i.e. tissue form healthy, non-tumour bearing mice was used as negative control, β-actin was used as a loading control.

The detailed methods are described in Example 1. The YB-1 specific antibody was obtained from Abeam, USA, the β-actin specific antibody was obtained from Abeam, USA.

The results are shown in Fig. 1.

As may be taken from Fig. 1 both glioma cells and glioma derived tumor stem cells express YB-1 under conditions of normoxia. In contrast thereto, normal brain tissue, i.e. brain tissue from mice not bearing any brain tumor do not express YB-1. Because of this, the molecular prerequisite for a replication of YB-1 dependent adenoviruses are given only in glioma cells and glioma derived tumor stem cells. Accordingly, those adenoviruses which replicate in a
YB-1 dependent matter will only replicate in glioma cells and glioma derived tumor cells, and will therefore only lyse glioma cells and glioma derived tumor stem cells but not normal cells from healthy tissue including non-tumor brain tissue.

**Example 3: Induction of cell death of glioma derived tumor tumor stem cells**

In this example, cell death of glioma derived tumor stem cells upon infection with an adenovirus which replicates in a YB-1 dependent manner was induced. The procedure was the one described in Example 1, i.e. the cytopathic effect (CPE) assay. Four glioma derived tumor stem cells which are also referred to as glioblastoma stem cells were used, namely R11.1, R28, R40 and R49 (Lottaz C, Cancer Res; 70(5) March 1, 2010; published online first on February 9, 2010). The adenovirus was delo3RGD.

The results and more specifically the cytopathic effect of AdDelo3RGD (also referred to herein as Delo3RGD) are shown in Fig. 2 (Control: untreated; TMZ: Temozolomide 100µM; AdEl -minus: Adenovirus replication deficient; wtAd: wild type adenovirus; Ad-Delo3RGD: YB-1 dependent oncolytic adenovirus). (It is to be acknowledged that the terms glioma derived tumor stem cell and glioblastoma stem cells are used in a synonymous manner herein if not specifically indicated differently.) As is evident from Fig. 2, wild type adenovirus and Ad-Delo3RGD are capable of killing brain tumor stem cells indicated by the lysis of neurospheres. The cytopathic effect which is indicative of cell killing is clearly more pronounced in case of treatment of tumor stem cells with DeloRGD compared to the treatment with Temozolomide (TMZ) which is the gold standard in the therapy of glioma. The E1-minus adenovirus (AdEl -minus) is not effective at all in mediating any cytopathic effect as its replication-deficiency due to deletion of the entire E1-region (E1A and E1B) is much more distinct as compared to DeloRGD.
**Example 4:** Treatment of mice bearing a tumor grown from glioma tumor stem cells R28 using a YB-1 dependent adenovirus

An orthotopic mouse model was established using glioma derived tumor stem cells as described in Example 1 herein. The mice were treated with various regimens, namely adenovirus delo3RGD alone, adenovirus delo3RGD together with temozolomide, temozolomide alone and phosphate buffered saline (PBS) as described in Example 1.

The results are shown in Fig. 3.

Fig. 3 is a diagram showing the survival rate of mice after implantation of glioma stem cells into the brain. From this diagram it is evident that adenovirus delo3RGD either alone or in combination with temozolomide has a clearly superior effect compared to the treatment with temozolomide alone, whereby temozolomide is regarded the gold standard in the chemotherapy of glioma. Accordingly, adenovirus delo3RGD is an effective agent for the killing of tumor stem cells and thus for the treatment of tumors and thus cancers which involve or contain tumor stem cells.

**Example 5:** Replication of a YB-1 dependent adenovirus under conditions of normoxia and hypoxia in tumor cells and tumor stem cells

This experiment discloses the replication of a YB-1 dependent adenovirus, namely delo3RGD, in tumor cells and tumor stem cells under conditions of normoxia and hypoxia.

The specific procedure is described in Example 1. As a measure for viral replication quantitative real-time PCR of adenoviral hexon DNA was performed. Conditions of hypoxia were such that the oxygen partial pressure was about 1 % and conditions of normoxia were such that the oxygen partial pressure was about 20 %. Expression of β-actin was used for normalizing the values and the expression of the wild type adenovirus under conditions of normoxia and hypoxia. Tumor cells were glioma cells U 87 and U 373, and tumor stem cells were glioma derived tumor stem cells R11.
The results are shown in Fig. 4 for glioma cells U 373, in Fig. 5 for glioma cells U 87 and in Fig. 6 for glioma derived tumor stem cells R40.

As is evident from Figs. 4 and 5 the replication of wild type adenoviruses decreases under hypoxic conditions compared to normoxic conditions in both glioma cells U87 and U373. In contrast thereto, the replication of adenovirus delo3RGD under hypoxic conditions increases compared to normoxic conditions.

The same is basically also true for the replication in glioma derived tumor stem cells R40 as depicted in Fig. 6. Again, the replication of adenovirus of the wild type is significantly decreased under conditions of hypoxia, whereby such decrease in replication is even more pronounced in tumor stem cells compared to the decrease under hypoxic conditions in tumor cells. In contrast thereto, the replication of both adenovirus of the wild type and delo3RGD is about the same in tumor stem cells R40 under both conditions of normoxia and hypoxia.

**Example 6: Efficient Cell Killing of Brain Tumor Initiating Cells by Ad-Delo3-RGD determined by Clonogenic Dilution Assay**

Brain Tumor Initiating Cells (BTIC:R11, R28, R40) were obtained from patients with primary glioblastoma as previously described (Beier D, Hau P, Proescholdt M et al. CD133+ and CD133- glioblastoma derived cancer stem cells show differential growth characteristics and molecular profiles. Cancer Research 2007; 67: 4010-4015) and were maintained as neurospheres in stem cell-permissive DMEM-F12 medium supplemented with 20 ng/mL of each human recombinant epidermal growth factor, human recombinant basic fibroblast growth factor (R & D Systems, Minneapolis, MN), human leukemia inhibitory factor (Chemicon, Schwalbach, Germany), and 2% B27 (Life Technologies, Carlsbad, CA). R11, R28 and R40 cells were seeded in 12-well plates (1 x 10^5 cells in 0.5 ml stem cell medium per well) and infected the next day with indicated virus or treated with TMZ. Cells were cultivated for further 24-36 hr under normoxic or hypoxic conditions (<0.66% O2). For the clonogenic assay, 24 h post Normoxia/Hypoxia, cells from each well of the 12 well-plates
were diluted into 24 well plates containing 1ml of stem cell medium in 1:10 dilution steps and
incubated for 4-6 weeks until medium of untreated cells turned into yellow.

The results are shown in Fig. 7. As may be taken therefrom, the clonogenic survival assay
demonstrates sustained killing of brain tumor initiating cells. (Control: untreated; TMZ:
Temozolomide 100µM; AdEl -minus: Adenovirus replication deficient (negative control);
wtAd: wild type adenovirus; Ad-Delo3RGD: YB-1 dependent oncolytic adenovirus. Red/dark
colour: Red/dark colour indicates no metabolic activity due to low cell survival. Yellow/light
colour: High metaboloc activity due to treatment failure, high cell survival.

**Example 7:** Ad-Delo3RGD replicates in Glioma Cancer Stem Cells as verified by Real-
Time PCR

Total DNA from cells from a 6 well was isolated using digestion buffer (100mM NaCl,
10mM Tris pH 8.0, 25mM EDTA pH 8.0, 0.5% SDS), Proteinase K and phenol-chloroform
isolation. After precipitation with ethanol, DNA was solubilised in 10 mM TrisCl pH 8.0. 100
ng of total DNA was used for RT-PCR. Quantitative real-time PCR was performed with the
ABI Prism 7900HT sequence detection system (Applied Biosystems, Foster City, CA) using a
SYBR-green fluorescent dye (Agilent Technologies, Boeblingen, Germany). The specific
primers (Eurofins mwg/operon, Germany) were fiber-fw: 5'-
AAGCTAGCCCTGCAAACATCA -3' (SEQ ID NO: 5), fiber-rev: 5'-
CCCAAGCTACCAGTGGCAGTA -3' (SEQ ID NO: 6), β-actin-fw: 5'-
TAAAGTAGGTGCACAGTAGGTCTGA-3' (SEQ ID NO: 3), and β-actin-rev: 5'-
AAAGTGCAAAAGAACACGGCTAAG-3' (SEQ ID NO: 4). Cycling conditions started with
initial enzyme activation at 95°C for 15 min, followed by 40 cycles of 15 sec denaturation at
95°C, 15 sec annealing at 60°C, and 15 sec elongation at 72°C. Homogeneity of amplification
product was confirmed by melting curve analysis (Tm-hexon: 85°C, Tm-B-actin: 83°C).

The results are shown in Fig. 8.
As shown in Fig. 8 Ad-Delo3RGD replicates around 100-600-fold better than the replication-deficient virus E1-minus adenovirus dl703, and even 4.3 fold better than wild type adenovirus in R40 cells. Values are given as fold increase of adenoviral fiber copies as determined after 72 hours and normalized to the increase of adenoviral fiber copies as observed after 4 hours.

**Example 8:** Ad-Delo3RGD causes down-regulation of CD44 expression determined by Western Blot Analysis

72h post infection with indicated virus R28 cells were lysed using ProteoJET Mammalian Cell Lysis Reagent (Fermentas) supplemented with complete protease inhibitor cocktail (Roche) and incubated at room temperature for 10 minutes. The lysates were clarified by centrifugation and protein concentration was measured using the bradford assay. SDS-sample buffer added and thirty microgram of cell extracts were resolved on SDS-polyacrylamide gels and transferred onto polyvinylidinediflouride membrane (Millipore). For detection of CD44 and tubulin the following antibodies were used: Anti-a-Tubulin mouse mAb (DM1A) from Calbiochem and rabbit monoclonal to CD44 (EPR1013Y or ab51037) from Abeam, USA. Immunoreactive proteins were detected using the enhanced chemiluminescence (ECL) or ECL plus western blot detection system (Amersham, Germany).

CD44 is, for example, described in Zoller M. "CD44: can a cancer-initiating cell profit from an abundantly expressed molecule?" Nature Reviews 2011, 11: 255-267.

The result is shown in Fig. 9.

Fig. 9 shows that CD44 protein expression was strongly inhibited in Ad-DEL03RGD infected R28 brain tumor initiating cells. AdEl -minus infection causes no inhibition of CD44 expression. wtAd serve as positive control. 1: Control (no treatment); 2: AdEl -minus; 3: Ad-Delo3RGD; 4: wtAd
The features disclosed in the preceding description, the claims as well as the figures may be individually or in any combination essential for the practising of the invention in its various embodiments.

2. The YB-1 dependent virus according to claim 1, wherein the tumor stem cells are part of a tumor.

3. The YB-1 dependent virus according to claim 2, wherein the tumor stem cells are part of a hypoxic area of the tumor or are contained in a hypoxic area of the tumor.

4. The YB-1 dependent virus according to any one of claims 1 to 3, wherein the virus is administered to the tumor, preferably into a hypoxic area, more preferably into the hypoxic area of which the tumor stem cells are part of or into the hypoxic area in which the tumor stem cells are contained.

5. A YB-1 dependent virus for use in the treatment of a tumor, wherein the treatment comprises the killing of tumor stem cells.

6. The virus according to claim 5, wherein the tumor stem cells are related to the tumor to be treated.

7. The virus according to any one of claims 5 and 6, wherein the tumor stem cells are part of a tumor.

8. The YB-1 dependent virus according to claim 7, wherein the tumor stem cells are part of a hypoxic area of the tumor or are contained in a hypoxic area of the tumor.

9. The YB-1 dependent virus according to any one of claims 5 to 8, wherein the virus is administered to the tumor, preferably into the hypoxic area.
10. A YB-1 dependent virus for use in a method for intratumorally administering a virus.

11. The virus according to claim 10, wherein the virus to be administered is the YB-1 dependent virus.

12. The virus according to any one of claims 10 to 11, wherein the virus is administered to a hypoxic area of the tumor.

13. The virus according to any one of claims 1 to 12, wherein the virus is an adenovirus.

14. The virus according to any one of claims 1 to 13, wherein the virus is an adenovirus different from wild type adenovirus.

15. The virus according to any one of claims 1 to 14, wherein the virus requires YB-1 for replication.

16. The virus according to any one of claims 1 to 15, wherein the virus replicates in cells containing YB-1.

17. The virus according to any one of claims 15 to 16, wherein the YB-1 is contained in the nucleus of the cells, preferably independent of the cell cycle.

18. The virus according to any one of claims 15 to 16, wherein the YB-1 is contained in the cytoplasm of the cells.

19. The virus according to any one of claims 15 to 18, preferably according to claim 18, wherein the YB-1 is deregulated YB-1.

20. The virus according to claim 19, wherein the deregulated YB-1 is acetylated or phosphorylated YB-1.
21. The virus according to claim 19, wherein the deregulated YB-1 is YB-1 which is overexpressed in the cell, preferably a tumor cell and more preferably a tumor stem cell.

22. The virus according to claim 21, wherein the YB-1 is over-expressed in the cell compared to a cell of the same type but not being a tumor cell.

23. The virus according to any one of claim 1 to 22, wherein the tumor stem cells contain YB-1.

24. The virus according to claim 23, wherein the YB-1 is contained in the nucleus of the tumor stem cells, preferably independent of the cell cycle.

25. The virus according to any one of claims 23 to 24, wherein the YB-1 is contained in the cytoplasm of the tumor stem cells.

26. The virus according to any one of claims 23 to 25, preferably according to claim 25, wherein the YB-1 is deregulated YB-1.

27. The virus according to claim 26, wherein the deregulated YB-1 is acetylated or phosphorylated YB-1.

28. The virus according to claim 26, wherein the deregulated YB-1 is YB-1 which is over-expressed in the cell, preferably a tumor cell and more preferably a tumor stem cell.

29. The virus according to claim 28, wherein the YB-1 is over-expressed in the tumor stem cell compared to a cell of the same type but not being a tumor cell, preferably not being a tumor stem cell.

30. The virus according to any one of claims 1 to 29, wherein the virus does not replicate in cells not containing YB-1, preferably YB-1 as defined in any of the preceding claims, but replicates in cells containing YB-1, preferably YB-1 as defined in any of the preceding claims.
31. The virus according to any one of claims 1 to 30, wherein a or the hypoxic region is a
region where the oxygen partial pressure is equal to or less than 4% \( p_{O_2} \), preferably equal to
or less than 2% \( p_{O_2} \) and more preferably equal to or less than 1% \( p_{O_2} \).

32. The virus according to any one of claims 1 to 31, wherein the virus is an oncolytic
adenovirus.

33. The virus according to any of claims 1 to 32, whereby the virus, preferably an
adenovirus, is replication deficient in cells which lack YB-1 in the nucleus, and whereby the
virus encodes an oncogene or oncogene product, in particular an oncogene protein, which
transactivates at least one viral gene, preferably an adenoviral gene, whereby the gene is
selected from the group comprising ElB55kDa, E4orf6, E4orf3 and E3ADP.

34. The virus according to claim 33, characterised in that the virus, in particular the
adenovirus replicates in cells which have YB-1 in the nucleus.

35. The virus according to any of claims 33 to 34, characterised in that the viral oncogene
protein is E1A and/or the oncogene is the gene coding for E1A and/or the oncogene protein
E1A.

36. The virus according to claim 35, characterised in that the viral oncogene protein E1A
is capable of binding a functional Rb tumor suppressor gene product.

37. The virus according to claim 35, characterised in that the viral oncogene protein E1A
is incapable of binding a functional Rb tumor suppressor gene product.

38. The virus according to any of claims 35 to 33, characterised in that the viral
oncoprotein E1A does not induce the localisation of YB-1 into the nucleus.

39. The virus according to any of claims 33 to 38, characterised in that the medicament is
for patients whose cells are Rb positive or Rb negative.
40. The virus according to any of claims 33 to 39, characterised in that the cells are Rb negative and the cell nucleus is YB-1 positive, preferably YB-1 positive in the nucleus independent from the cell cycle.

41. The virus according to any of claims 33 to 40, characterised in that the cells are p53 positive or p53 negative.

42. The virus according to any of claims 33 to 41, characterised in that the oncogene protein exhibits one or several mutations or deletions compared to the wildtype oncogene protein E1A, whereby the deletion is preferably one selected from the group comprising deletions of the CR3 stretches and deletions of the N-terminus and deletions of the C-terminus.

43. The virus according to claim 42, characterised in that the E1A oncogene protein is capable of binding to Rb.

44. The virus according to any of claims 33 to 41, characterised in that the oncogene protein comprises one or several mutations or deletions compared to the wildtype oncogene protein, whereby the deletion is preferably a deletion in the CR1 region and/or CR2 region.

45. The virus according to claim 44, characterised in that the oncogene protein E1A is incapable of binding to Rb.

46. The virus according to any of claims 33 to 45, characterised in that the viral oncogene protein, preferably E1A, is under the control of a tissue- and/or tumor-specific promoter.

47. The virus according to any of claims 33 to 46, characterised in that the virus, particularly the adenovirus, codes for YB-1.

48. The virus according to claim 47, characterised in that YB-1 is under the control of a tissue-specific and/or tumor-specific promoter.
49. The virus according to any of claims 33 to 48, characterised in that the virus, preferably the adenovirus, codes for at least one protein, whereby the protein is selected from the group comprising E4orf6, E4orf3, ElB55k and adenoviral E3ADP protein.

50. The virus according to any of claims 33 to 49, characterised in that the cells comprise YB-1 in the nucleus, preferably that the cells forming the tumor or part thereof have YB-1 in the nucleus.

51. The virus according to any of claims 33 to 50, characterised in that the tumor comprises YB-1 in the nucleus after induction of the transport of YB-1 into the nucleus.

52. The virus according to claim 51, characterised in that the transport of YB-1 into the nucleus is triggered by at least one measure selected from the group comprising irradiation, administration of cytostatics and hyperthermia.

53. The virus according to claim 52, characterised in that the measure is applied to a cell, an organ or an organism, preferably an organism in need thereof, more preferably an organism suffering from said disease.

54. The virus according to any of claims 33 to 53, characterised in that the virus, preferably the adenovirus, is selected from the group comprising AdA24, dl922-947, ElAd/01/07, dil 119/1 131, CB 016, dl520 and viruses lacking an expressed viral oncogene which is capable of binding a functional Rb tumor suppressor gene product.

55. The virus according to any of claims 33 to 54, characterised in that the virus, preferably the adenovirus, is designed such that the replication is controlled by YB-1 through the activation of the E2-late promoter, preferably the activation is predominantly controlled through the activation of the E2-late promoter.

56. The virus according to any of claims 33 to 55, whereby the virus comprises a nucleic acid coding for a transgene.
57. The virus according to any of claims 33 to 55, whereby the virus comprises the translation and/or transcription product of a transgene.

58. The virus according to any of claims 56 to 57, whereby the nucleic acid comprises a transgene or a nucleic acid coding for a transgene.

59. The virus according to any of claims 56 to 58, whereby the transgene is selected from the group comprising prodrug genes, cytokines and genes for cytokines, apoptosis-inducing genes, tumor suppressor genes, genes for metalloproteinase inhibitors and genes for angiogenesis inhibitors.

60. The virus according to any of claims 56 to 59, whereby the transgene is selected from the group comprising nucleic acids for siRNA, for aptamers, for antisense molecules and for ribozymes, whereby the siRNA, the aptamer, the antisense molecule and/or the ribozyme are targeting a target molecule.

61. The virus according to claim 60, whereby the target molecule is selected from the group comprising resistance relevant factors, anti-apoptosis factors, oncogenes, angiogenesis factors, DNA synthesis enzymes, DNA repair enzymes, growth factors, receptors for growth factors, transcription factors, metalloproteinases, preferably matrix metalloproteinase kinases, and plasminogen activator of the urokinase type.

62. The virus according to any one of claims 1 to 32, wherein the virus is E1A12S positive but lacking a functionally active E1A13S.

63. The virus according to any of claims 1 to 32, whereby the virus, preferably the adenovirus, expresses a first protein which is selected from the group comprising an E1B protein and an E4 protein, prior to a second protein which is selected from the group comprising an E1A-protein.
64. The virus according to claim 63, characterised in that the first protein is an EIB protein, preferably an ElB55kd protein.

65. The virus according to claim 63, characterised in that the first protein is an E4 protein, preferably an E4orf6 protein.

66. The virus according to any of claims 63 to 65, characterised in that the first protein is a combination of EIB protein and E4 protein, preferably a combination of ElB55kD protein and E4orf6 protein.

67. The virus according to any of claims 63 to 66, characterised in that the E1A protein is an E1A12S protein.

68. The virus according to any of claims 1 to 32 and/or preferably an adenovirus as defined in any of claims 63 to 67, characterised in that the virus comprises at least one nucleic acid coding for a protein which is selected from the group comprising EIB proteins, E4 proteins and E1A proteins, whereby the at least one protein is under the control of a promoter which is different from the promoter controlling the expression of the protein in a wildtype adenovirus.

69. The virus according to claim 68, characterised in that the at least one protein is an EIB protein, preferably an ElB55kD protein.

70. The virus according to claim 68 or 69, characterised in that the at least one protein is an E4 protein, preferably an E4orf6 protein.

71. The virus according to any of claims 68 to 70, characterised in that the at least one protein is an E1A protein, preferably an E1A12S protein.

72. The virus according to any of claims 68 to 71, characterised in that the at least one protein is a combination of EIB protein and E4 protein, preferably a combination of ElB55kD protein and E4orf6 protein.
73. The virus according to any of claims 68 to 71, characterised in that the at least one protein is a combination of EIB protein and E1A protein, preferably a combination of ElB55kD protein and E1A12S protein.

74. The virus according to any of claims 68 to 71, characterised in that the at least one protein is a combination of E4 protein and E1A protein, preferably a combination of E4orf6 protein and E1A12S protein.

75. The virus according to any of claims 68 to 71, characterised in that the at least one protein is a combination of EIB protein, E4 protein and E1A protein, preferably a combination of ElB55kD protein, E4orf6 protein and E1A12S protein.

76. The virus according to any of claims 68 to 75, characterised in that the expression of the EIB protein is controlled by a promoter, whereby the promoter is selected from the group comprising tumor-specific promoters, organ-specific promoters, tissue-specific promoters, heterologous promoters and adenoviral promoters, whereby the adenoviral promoter is different from the EIB promoter.

77. The virus according to any of claims 68 to 76, characterised in that the expression of the E4 protein is controlled by a promoter, whereby the promoter is selected from the group comprising tumor-specific promoters, organ-specific promoters, tissue-specific promoters, heterologous promoters and adenoviral promoters, whereby the adenoviral promoter is different from the E4 promoter.

78. The virus according to claim 76 and 77, whereby the adenoviral promoter is the E1A promoter.

79. The virus according to any of claims 68 to 78, characterised in that the expression of the E1A protein is controlled by a promoter, whereby the promoter is selected from the group comprising tumor-specific promoters, organ-specific promoters, tissue-specific promoters,
heterologous promoters and adenoviral promoters, whereby the adenoviral promoter is different from the E1A promoter.

80. The virus according to any of claims 76 to 79, characterised in that the expression of the E1A protein is YB-1 controlled or can be regulated by YB-1.

81. The virus according to any of claims 76 to 80, characterised in that the promoter controlling the expression of the E1A protein is the adenoviral E2 late promoter.

82. The virus according to any of claims 63 to 81, characterised in that the E4 protein, preferably the E4orf6 protein, and the EIB protein, preferably the EIB55kd protein, are under the control of the same or a common promoter.

83. The virus according to any of claims 1 to 32, and/or preferably according to any of claims 63 to 82, characterised in that the virus provides YB-1 in the nucleus through at least one adenoviral protein or mediates the provision of YB-1 in the nucleus through at least one adenoviral protein, whereby preferably the adenoviral protein is different from E1A.

84. The virus according to any of claims 1 to 32, and/or preferably according to any of claims 63 to 83, characterised in that the virus provides YB-1 for adenoviral replication through at least one adenoviral protein or mediates the provision of YB-1 for adenoviral replication through at least one adenoviral protein, whereby preferably the adenoviral protein is different from E1A.

85. The virus according to claim 83 or 84, characterised in that the adenoviral protein is a complex of E4orf6 and EIB55kd.

86. The virus according to any of claims 1 to 32, and/or preferably according to claims 63 to 85, characterised in that the nucleic acid of the adenovirus comprises at least one functionally inactive adenoviral region, whereby the region is selected from the group comprising the E1 region, the E3 region, the E4 region and combinations thereof.
87. The virus according to claim 86, characterised in that the region is the E1 region.

88. The virus according to claims 86 or 87, characterised in that the region is the E3 region.

89. The virus according to any of claims 86 to 88, characterised in that the region is the E4 region.

90. The virus according to any of claims 87 to 89, characterised in that the region comprises the E1 region, the E3 region and the E4 region.

91. The virus according to any of claims 1 to 32, and/or preferably to any of claims 63 to 90, characterised in that the virus comprises at least one expression cassette, whereby the expression cassette comprises at least one promoter and a nucleic acid coding for an adenoviral protein, whereby the adenoviral protein is an E1B protein, preferably an E1B55kD protein.

92. The virus according to claim 91, characterised in that the promoter is different from the E1B promoter.

93. The virus according to claim 92, characterised in that the promoter is selected from the group comprising tumor-specific promoters, organ-specific promoters, tissue-specific promoters, heterologous promoters and adenoviral promoters, whereby the promoter is different from the E1B promoter.

94. The virus according to any of claims 1 to 32, and/or preferably according to any of claims 63 to 93, characterised in that the virus comprises at least one expression cassette, whereby the expression cassette comprises at least one promoter and a nucleic acid coding for an adenoviral protein, whereby the adenoviral protein is an E4 protein, preferably an E4orf6 protein.
95. The virus according to claim 94, characterised in that the promoter is different from the E4 promoter.

96. The virus according to claim 95, characterised in that the promoter is selected from the group comprising tumor-specific promoters, organ-specific promoters, tissue-specific promoters, heterologous promoters and adenoviral promoters, whereby the adenoviral promoter is different from the E4 promoter.

97. The virus according to any of claims 93 to 96, characterised in that the promoter is the E1A promoter.

98. The virus according to any of claims 1 to 32, and/or preferably according to any of claims 63 to 97, characterised in that the virus comprises at least one expression cassette, whereby the expression cassette comprises at least one promoter and a nucleic acid coding for an adenoviral protein, whereby the adenoviral protein is an E1A protein, preferably an E1A12S protein.

99. The virus according to claim 98, characterised in that the promoter is different from the E1A promoter.

100. The virus according to claim 99, characterised in that the promoter is selected from the group comprising tumor-specific promoters, organ-specific promoters, tissue-specific promoters, heterologous promoters and adenoviral promoters.

101. The virus according to any of claims 63 to 100, characterised in that the adenovirus comprises a nucleic acid, whereby the nucleic acid codes for YB-1.

102. The virus according to claim 101, characterised in that the nucleic acid coding for YB-1 is under the control of a promoter, whereby the promoter is preferably the E2 late promoter.
103. The virus according to claim 101 or 102, characterised in that the nucleic acid coding for YB-1 is under the control of a promoter, whereby the promoter is YB-1 dependent and YB-1 controlled, respectively.

104. The virus according to any of claims 101 to 103, characterised in that the nucleic acid coding for YB-1 is part of the expression cassette comprising a nucleic acid coding for an E1A protein, preferably a nucleic acid coding for an E1A12S protein.

105. The virus according to claim 104, characterised in that the nucleic acid coding for the E1A protein is separated from the nucleic acid coding for YB-1 through an IRES sequence.

106. The virus according to any of claims 93 to 105, characterised in that the nucleic acid coding for the E4 protein, preferably the E4orf6 protein, and the nucleic acid coding for the E1B protein, preferably the EIB55kD protein, are contained in an expression cassette, whereby preferably the two coding sequences are separated through an IRES sequence.

107. The virus according claim 106, characterised in that the promoter of the expression cassette is selected from the group comprising tumor-specific promoters, organ-specific promoters, tissue-specific promoters, heterologous promoters and adenoviral promoters, whereby the adenoviral promoter is different from the E4 promoter and different from the E1B promoter, preferably different from the wildtype E4 promoter and different from the wildtype E1B promoter.

108. The virus according to any of claims 63 to 107, characterised in that the virus comprises an expression cassette comprising a promoter and a nucleic acid sequence, whereby the nucleic acid sequence is selected from the group comprising aptamers, ribozymes, aptazymes, antisense molecules and siRNA.

109. The virus according to any of claims 63 to 107, characterised in that the virus comprises an expression cassette comprising a promoter and a nucleic acid sequence, whereby the nucleic acid sequence is a coding nucleic acid, whereby the nucleic acid codes for a
molecule which is selected from the group comprising peptides, polypeptides, proteins, anticalines, antibodies and antibody fragments.

110. The virus according to any of claims 63 to 107, characterised in that the virus comprises an expression cassette, whereby the expression cassette comprises a promoter and a nucleic acid sequence, whereby the nucleic acid sequence is selected from the group comprising apoptosis inducing genes, prodrug genes, protease inhibitors, tumor suppressor genes, cytokines and angiogenesis inhibitors.

111. The virus according to any of claims 1 to 32, 33 to 62 and 63 to 110, characterised in that the virus is a recombinant adenovirus.

112. The virus according to any of claims 1 to 32, 33 to 62 and 63 to 111, characterised in that the virus is an adenovirus mutant.

113. The virus according to any of claims 1 to 32, 33 to 62 and 63 to 112, characterised in that the virus is replication deficient.

114. The virus according to claim 113, characterised in that the virus is capable of replicating in cells comprising deregulated YB-1 or having YB-1 in the nucleus.

115. The virus according to claim 114, characterised in that the cells contain YB-1 in the nucleus independent of the cell cycle.

116. The virus according to any one of claims 1 to 115, wherein the tumor is a solid tumor.

117. A nucleic acid coding for a virus according to any one of claims 1 to 116 for use in any method as defined in any of the preceding claims.

118. A vector comprising the nucleic acid according to claim 117.
119. A cell comprising a nucleic acid according to claim 117 or a vector according to claim 118.

120. The cell according to claim 119, whereby such cell is different from a human or a human embryonic stem cell.

121. A pharmaceutical composition comprising a virus according to any one of claims 1 to 116.

122. A pharmaceutical composition comprising a nucleic acid according to claim 117.

123. A method for the killing of tumor stem cells comprising the administration of a virus as defined in any one of claims 1 to 116 to a tumor containing tumor stem cells or suspected of containing tumor stem cells.

124. A method for intratumorally administering a virus comprising the step of administering the virus intratumorally into a tumor, wherein the virus is a virus as defined in any one of claim 1 to 116.

125. The method according to any one of claims 123 to 124, wherein the virus is administered to or into a hypoxic area of the tumor.

126. The virus according to any one of claims 1 to 116 and the method according to any one of claims 123 to 125, wherein the tumor comprises cells expressing YB-1 and/or CD44.

127. The virus according to any one of claims 1 to 116 and 126 and the method according to any one of claims 123 to 126, wherein the tumor is a solid tumor and preferably the tumor is selected from the group comprising lymphoma, glioma, hepatocellular carcinoma, breast cancer, lung cancer, ovarian cancer, synovial sarcoma, melanoma, prostate cancer, colorectal cancer, head and neck cancer, pancreatic cancer and urothelial carcinoma.
Western Blot Analysis of YB-1 Expression in glioma cells, glioma derived tumor stem cells and normal tissue

Fig. 1

Normal Brain Tissue

Tumor stem cells: R11, R28, R49

Glioma cells: U87, U373

YB-1
β-actin

U87 U373 R11 R28 R49
Efficient Cell Killing of Brain Tumor Initiating Cells by Ad-Delo3RGD

Fig. 2
Fig. 3

Survival rate of mice after implantation of glioma stem cells R28 into the brain

-PBS
-Temozolomide
-Delo3RGD
-Delo3RGD + Temozolomide

Days
Fig. 4 Quantitative real-time PCR of adenovirus hexon DNA in U373 cells under normoxia and hypoxia conditions.
Fig. 5  Quantitative real-time PCR of adenovirus hexon DNA in U87 cells under normoxia and hypoxia conditions
Fig. 6  Quantitative real-time PCR of adenovirus hexon DNA in the glioma cancer stem cell R40 under normoxia and hypoxia conditions.

![Bar graph showing copies of hexon/β-actin under normoxia and hypoxia conditions for Delo3-RGD and wt constructs.](image-url)
Efficient Cell Killing of Brain Tumor Initiating Cells by Ad-Delo3-RGD determined by Clonogenic Dilution Assay

Fig. 7
Fig. 9

Western Blot Analysis of CD44 Expression in Brain Initiating Cells 72 h Post Infection

1: Control (no treatment); 2: AdE1-minus; 3: Ad-Dele3RGD; 4: wtAd